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Cross-Reactivity of Plant and Animal Allergens

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Introduction

This review will address cross-reactivity of pollen, fungal, and animal aeroallergens. In the 15 years or so that have transpired since similar reviews on pollen cross-reactivity were published, significant additional information both on cross-allergenicity and on plant systematics has become available *(1,2).* This review will attempt to incorporate those advances. Cross-reactivity among foods will only be discussed in the context of relationships to pollen allergens. Likewise, cross-reactivity of pharmaceuticals or latex will not be discussed.

Clinical Implications

Cross-reactivity is the ability of an antigen to bind with an antibody that was raised to a different antigen. It may arise by one of two mechanisms: shared epitopes on multivalent antigens, in which case antibodies should bind with the same affinity; or conformational similarity of epitopes, in which case antibodies would bind with lesser affinity. Aalberse has recently reviewed factors that affect allergenicity and cross-reactivity, pointing out the importance of similarities owing to not only primary but also tertiary structure *(3).* Despite amino acid homology for epitope segments, differences in tertiary folding may result in demonstrably different epitopes.

Why should cross-reactivity be of concern to the practicing allergist? It is because such relationships will impact both on diagnosis and therapy of inhalant allergy. For example, skin test positivity to a nonendemic plant may be explained either by prior exposure in a

Table 1
Methods to Determine Cross-Reactivity

Nonimmunologic assessment
Isoelectric focusing (IEF)
Polyacrylamide gel electrophoresis (PAGE)
Amino acid sequencing
Gene sequencing
Antigenic assessment
Gel double immunodiffusion with animal antisera
Passive hemagglutination and inhibition with animal antisera
Crossed immunoelectrophoresis (CIE)
Immunoblot with monoclonal antibodies
Allergenic assessment
Skin test comparison
Prausnitz-Küstner neutralization or extinction
Leukocyte histamine release inhibition
Radioallergosorbent test (RAST) inhibition
Enzyme-linked immunosorbent assay (ELISA) inhibition
Crossed radioimmunoelectrophoresis (CRIE)
Immunoblot with IgE ELISA

mobile population or cross-reactivity with endemic plants. The decision whether to include such a nonendemic extract in an allergen immunotherapy formulation depends on several factors, one of which is whether related plants provide adequate coverage. At a time of constricting availability of particular plant vaccines, this issue becomes crucial. Additionally, as more information becomes known on optimal allergen dosing, cross-reactivity aspects will impact on formulation. Disregard of cross-reacting allergens may also increase the likelihood of adverse reactions owing to dosing with a greater amount of the same allergen than realized.

Methods of Assessing Cross-Reactivity

A variety of techniques have been utilized in the assessment of cross-reactivity, all of which have inherent strengths and weaknesses (*see* Table 1). These assays may measure different properties, often giving discrepant results, reminiscent of the parable of the blind men and the elephant. Generally, assays that evaluate IgG antisera (antigenicity) tend to emphasize differences, whereas IgE assays such as radioallergoabsorbent test (RAST) inhibition (allergenicity) tend to accentuate similarities. The choice of donors in establishing human antisera pools for such assays is critical, and may certainly affect the results of cross-reactivity studies. It is important to be mindful of these possible pitfalls in assessing results.

Nonimmunological Assessments

Proteins may be spatially separated by molecular weight or electrical charge, using polyacrylamide gel electrophoresis (PAGE) or isoelectric focusing (IEF), followed by appropriate staining. Further two-dimensional separation may be achieved by two-step procedures run at 90° angles. Such techniques have used to demonstrate potency (or absence or presence) of proteins in vaccine formulation, but do not provide by themselves any data concerning immunological activity. Likewise, amino acid sequencing and DNA-cloning results must be taken in combination with antisera determinations to truly assess antigenicity or allergenicity.

Cross-Antigenicity

Antigenic properties of pollen extracts have been studied by raising animal antisera. Ouchterlony passive double immunodiffusion was used to demonstrate "identity" or "nonidentity" of pollen extracts by Wodehouse in 1957 *(4).* However, much better separation of the precipitin bands was achieved with the use of crossed immunoelectrophoresis (CIE), or crossed-line immunoelectrophoresis (CLIE) *(5).* Multivalent allergens could then be demonstrated by overlying the gel with human antisera with either a radioisotope (CRIE) or enzyme marker. A less labor-intensive technique, which is now the preferred method, is immunoblotting: protein separation by sodium dodecyl sulfate (SDS)-PAGE or IEF, nitrocellulose transfer, followed by overlying with sera and a marker *(6,7).*

Cross-Allergenicity

Comparison of skin test reactivity is perhaps the oldest method of assessing cross-reactivity, as reported by Lamson in 1931 *(8).* More recently, statistical analyses of skin-test results were done by Weber utilizing Spearman rank correlations, and by Kurata and coworkers using Pearson coefficients *(9–11).* While high r values support allergenic similarity, this may also result from concomitant exposure. Extinction of skin test reactivity of the Prausnitz-Küstner reaction with inhibiting extracts, as done by Rackemann and Wagner in 1936, also gave clues to cross-reactivity of various pollens *(12).* However, results varied using different subjects, suggesting nonspecific intrinsic factors could color the outcome.

Perhaps the in vitro counterpart to P-K neutralization is RAST inhibition, or the easier modifications using enzyme or fluorescent labeling (enzyme-linked immunosorbent assay [ELISA] or FAST inhibition) *(13).* The use of varying inhibitor concentrations allows the development of inhibition curves, which allow the perception of both qualitative and

quantitative assessment. The banding observed with immunoblotting can also be modified by the use of inhibiting extracts, providing some evidence for cross-reactivity as well.

Amino acid sequencing or recombinant DNA studies have resulted in partial or complete identification of a number of allergenic proteins. Sequence homology implies complete cross-reactivity, although several studies have shown that this is not always the case. Despite high amino acid homology of the NH_2-terminal between Lol p 1 and Cyn d 1, cross-reactivity of IgG monoclonal antibodies (MAbs) and human IgE between Bermuda grass and perennial ryegrass is weak *(14)*.

Pollen Interrelationships

Despite recent progress, information on pollen cross-reactivity is fairly limited. Studies have not been performed for the majority of pollen vaccines used in practice. The most extensive research has been with northern pasture grass pollens, the major ragweeds, sages, cedars, and junipers, and with members of the closely related birch and beech botanical families. Preliminary work has been done with chenopod and amaranth weeds.

Plant Systematics

Because of this limited data, plant systematics has been used to infer the likelihood of cross-reactivity. The validity of this process depends on two premises. The first is that more closely related plants will have greater similarities and more shared antigens. The second premise is that the presently accepted botanical classification truly reflects phylogeny, i.e., two plants in the same genus evolved from a common ancestor, two in the same family from a more distant ancestor, and so on *(1)*. Therefore, two plants in the same genus would be expected to have the greatest number of shared allergens, those in the same family perhaps fewer, and so on. Distantly related plants would be expected to show little cross-reactivity. Research to date generally supports the use of this approach with some exceptions, the most notable being the presence of panallergens such as the profilins, which are pollen and food allergens, and may even be found in animal sources.

Older taxonomic schemes were based primarily on morphologic similarities, which were sometimes misleading because evolutionary drift could be along both divergent and convergent paths. The taxonomy utilized here is that of Judd and colleagues from their recently published text "Plant Systematics: A Phylogenetic Approach" *(15)*. In addition to morphology, supporting data is derived from embryology and palynology, as well as biochemical, chloroplast gene, chromosome, and DNA analyses *(16)*. Groupings of plants related by a

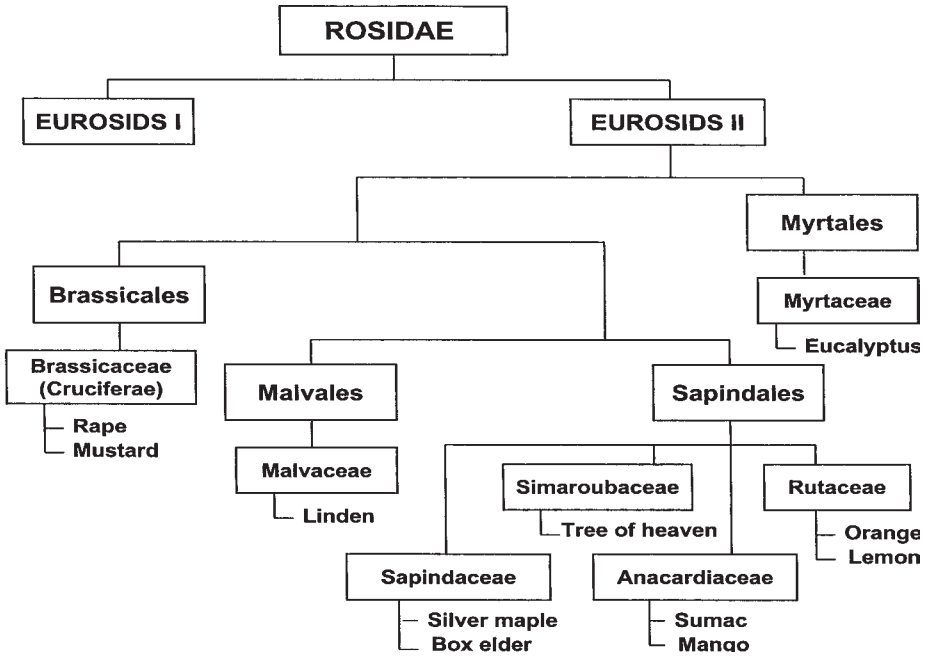

Fig. 1. Taxonomy of vascular plants. See text for details.

common ancestor are called "clades," and may be of any size, including even orders, superorders, or classes. In some cases, the evidence for monophyly (descent from a common ancestor) is quite strong, and one would in turn expect strong cross-reactivity.

Despite great recent advances, significant controversy still exists in the placement of a number of plant groups. Tracheophytes (vascular plants) have been grouped into spore forming plants, such as ferns and club mosses, and seed plants. The later group has traditionally been divided into the Gymnosperms and the Angiosperms (flowering plants). While the monophyly of angiosperms is strongly supported, that of the Gymnosperms is not. Of the four groups of Gymnosperms, cycads, gingkoes, and conifers are closely related, while Gnetophytes, the group containing *Ephedra* (Mormon tea), is more closely related to the Angiosperms. Additionally, the traditional division of flowering plants into Monocots and Dicots is likewise not supported by recent data: Monocots *are* monophylous, but Dicots are not. A large monophylous subset is the Tricolpates, also known as true dicots (Eudicots). This group is identified by pollen type (containing three apertures), and similarities of gene sequences *(15).* Other groupings within the "Dicots" are the Magnoliid complex and the Paleoherbs, the latter being more closed linked to the Monocots. (*See* Fig. 1 for clarification.) The following sections will expand on taxonomy as it applies to specific groups of relevant allergenic plants.

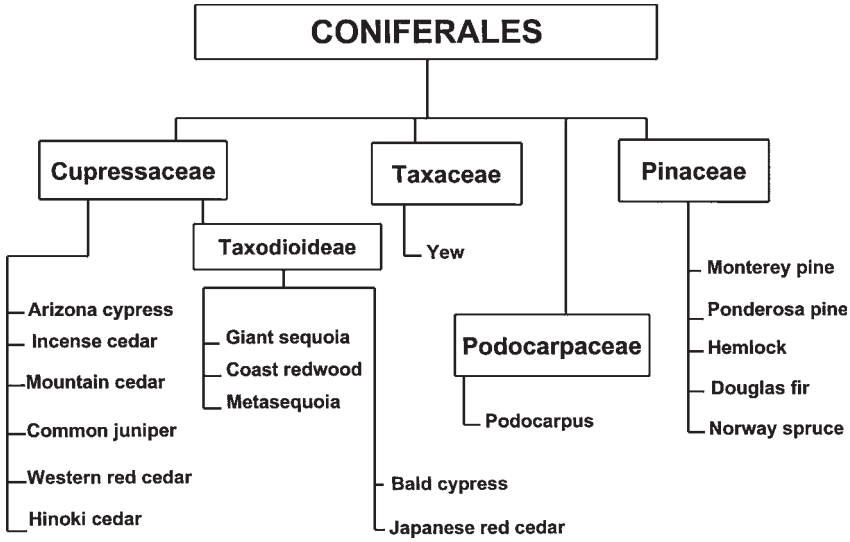

Fig. 2. Taxonomy of the Order Coniferales, with representative members of the families. See text for details.

Conifers

Among the evergreens of the order Coniferales, a large family is Cupressaceae. This family includes cedars, junipers, pfitzers (*Juniperus spp, Thuja spp*), and cypresses (*Cupressus spp*). Under the older classification there was a smaller family, Taxodiaceae, which included bald cypress, redwoods, sequoias, and a very important producer of pollinosis in Japan, Japanese red cedar (*Cryptomeria japonica*). This family has now been incorporated into Cupressaceae (*see* Fig. 2). In 1929, Black had demonstrated strong skin test correlations between mountain cedar (*J. ashei* or *J. sabinoides*) and a non-endemic conifer Port Orford cypress (*Chamaecyparis lawsonia*) *(17).* He also reported that mountain cedar hay fever sufferers derived good benefit from immunotherapy with Port Orford cypress vaccine. Members of Cupressaceae are strongly cross-reactive, with most studies demonstrating consistent cross-inhibition with both animal antisera and human IgE antibodies *(18–20).* Weber had shown strong skin test correlation between mountain cedar and red cedar (*J. virginiana*), but weaker correlations between these two junipers and a pine, *Pinus ponderosa (9).* Dirksen and Østerballe found no skin test correlation between juniper and two members of Pinaceae, pine, and spruce *(21).* Earlier data on "Taxodiaceae" suggested little cross-reactivity between members, with initial conflicting reports of Japanese red cedar cross-reactivity with Cupressaceae members. In 1975, Yoo and coworkers had examined 10 Cupressaceae members plus Japanese red cedar and coast redwood (*Sequoia sempervirens*) *(18).* They found strong cross-reactivity between the cypress family members, but

little between these and the other two. Nor did the redwood and Japanese red cedar cross-react. However, Yasueda and colleagues reported cross-allergenicity between *C. japonica* and a cypress, hinoki white cedar (*Chaemcyparis obtusa*) based on skin tests and RAST inhibition *(22).* Schwietz and colleagues investigated 12 members of Cupressaceae including the major tree allergen of south Texas, mountain cedar (*Juniperus ashei*), and Japanese red cedar (*Cryptomeria japonica*), as well as a pine family member, deodar cedar (*Cedrus deodora*), and an angiosperm, salt cedar (*Tamarix gallica*) *(23).* They found strong cross-reactivity between the major mountain cedar allergen, a 40 kD glycoprotein, with homologous allergens in the other family members, as well as the 46 kD major allergen of Japanese red cedar. Complementary DNA for Jun a 1 has recently been cloned and sequenced, with expression of the recombinant protein *(24).* The amino acid sequence showed high homology to Cry j 1 and Cha o 1, but Jun a 1 contained N-glycosylation sites distinct from those of Cry j 1. Tinghino and coworkers have reported an allergen from *J. oxycedrus*, Jun o 2, which belongs to a group of calcium-binding proteins, which also appear to be found in a diverse array of plants *(25).* Immunoblotting studies revealed inhibition by other family members: *J. ashei*, *C. arizonica*, and *C. sempervirens*. Additionally, the unrelated weed pellitory, *Parietaria judaica*, inhibited at low concentrations, while olive, Olea *europaea*, and ryegrass, *Lolium perenne*, inhibited at greater concentrations.

The pine family consists of pines (*Pinus spp*), spruces (*Picea spp*), hemlocks (*Tsuga spp*), and firs (*Abies spp, Pseudotsuga*). Fortunately, despite being produced in copious amounts, pine pollens are weak allergenically, and produce little significant hayfever. Some rapidly eluted proteins from Monterey pine (*Pinus radiata*) are able to modestly inhibit solid phase perennial ryegrass (*Lolium perenne*) RAST discs *(26).* Although this demonstration of cross-reactivity, however modest, between such distantly related species was perplexing at the time, this report probably reflects the ubiquity of certain minor allergens such as the profilins, which are discussed in greater detail in another section.

Grasses and Related Plants

Monocots of greatest interest to allergists lie within the superorder Commelinanae. One clade includes palms of the family Areceae. Another larger clade includes not only the grass family (Poaceae or Graminae) but grass allies such as rushes, sedges, and cattails (Juncaceae, Cyperaceae, and Typhaceae families, respectively). *See* Figs. 3 and 4 for clarification of botanical relationships.

Poaceae is a very large family with several subfamilies and a number of tribes. The subfamily Pooideae (Festucoideae) of temperate pasture grasses contains many prolific pollen producers divided among

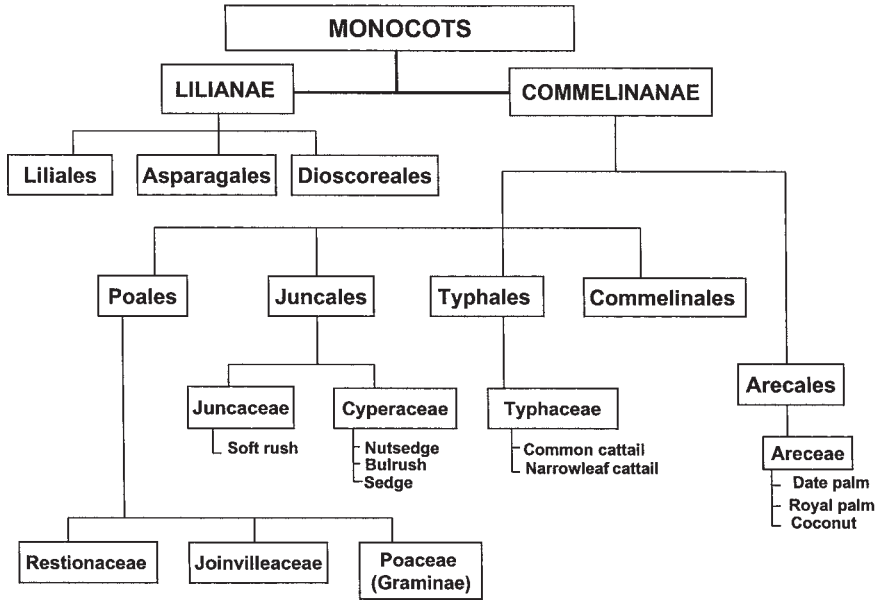

Fig. 3. Taxonomy of Monocot Clade, with representative allergenic plants. See text for details.

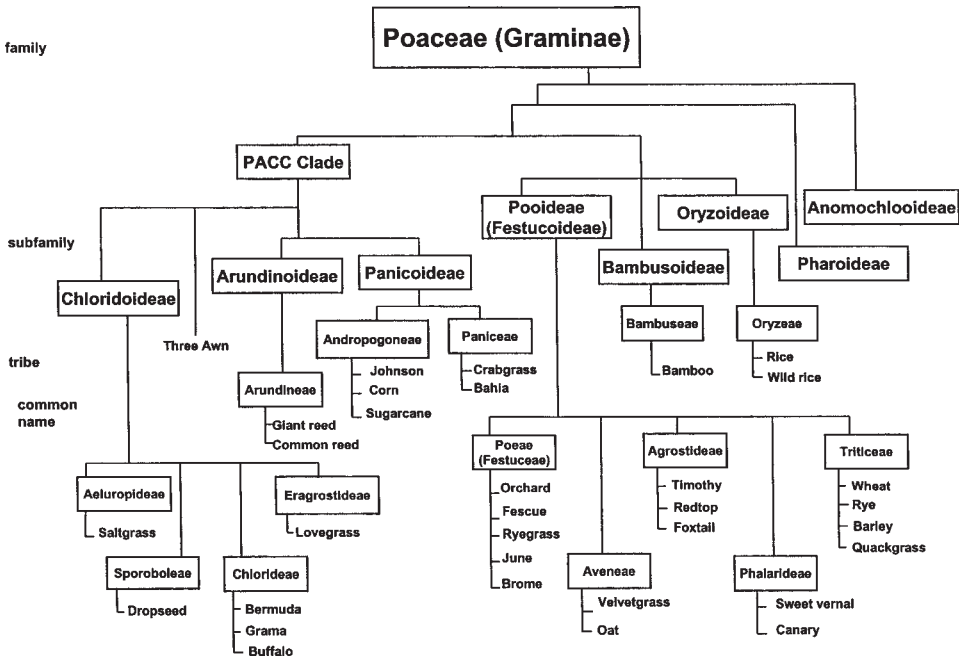

Fig. 4. Taxonomy of Grass Family, Poaceae, with representative grasses of allergenically important tribes. See text for details.

Table 2
Representative Allergenic Grasses of Poaceae (Graminae)

Subfamily	Tribe	Genus	Species	Common Name
Pooideae	Poeae	Poa	pratensis	Kentucky blue
(Festucoideae)	(Festuceae)	Festuca	elatior	Meadow fescue
		Lolium	perenne	Perennial ryegrass
		Dactylis	glomerata	Orchard
		Bromus	inermus	Smooth brome
	Aveneae	Avena	sativa	Oat
		Holcus	lanatus	Velvet, Yorkshire fog
	Phalarideae	Phalaris	canariensis	Canary
		Anthoxanthum	odoratum	Sweet vernal
	Agrostideae	Agrostis	alba	Redto
		Phleum	pratense	Timothy
	Triticeae	Triticum	aestivum	Wheat
		Secale	cereale	Rye
		Hordeum	vulgare	Barley
		Agropyron	smithii	Western wheatgrass
Bambusoideae	Bambuseae	Bambusa	bambos	Bamboo
Oryzoideae	Oryzeae	Oryza	sativa	Rice
		Zizania	aquatica	Wild rice
PACC CLADE		Aristida	longiseta	Red three-awn
Arundinoideae	Arundineae	Arundo	donax	Giant reed
		Phragmites	communis	Common reed
		Cortaderia	sellona	Pampasgrass
Panicoideae	Paniceae	Panicum	capillare	Witchgrass
		Paspalum	notatum	Bahia
	Andropogoneae	Sorghum	halepense	Johnson
		Sorghastrum	nutans	Indian grass
		Zea	mays	Corn
		Saccharum	officinarum	Sugarcane
Chloridoideae	Chlorideae	Chloris	verticillata	Windmill grass
		Cynodon	dactylon	Bermuda
		Bouteloua	gracilis	Blue grama
		Buchloe	dactyloides	Buffalo
	Aeluropideae	Distichlis	stricta	Saltgrass
	Sporoboleae	Sporobolus	cryptandrus	Sand dropseed

five tribes: Poeae, containing among others orchard, fescue, brome, june, and ryegrass; Aveneae with oat and velvet grass; Phalarideae with canary and sweet vernal; Agrostideae with timothy, redtop, and foxtail; and Triticeae with western wheatgrass and the cereal grains wheat, rye, and barley. The PACC clade contains the subfamilies Arundinoideae, Panicoideae, and Chloridoideae as well as arid grasses of the genus *Aristida* (three-awns). Panicoideae contains the tribes Paniceae with bahia and crabgrass, and Andropogoneae with johnson, indian grass, corn, and sugarcane. Chloridoideae has four tribes: Aeluropideae with saltgrass; Eragrostideae with lovegrass; Sporoboleae with

dropseed; and Chlorideae with windmill grass, bermuda, grama, and buffalo. *See* Table 2 for representative members.

Assessments of cross-reactivity within the grasses dates to the observation of Freeman that timothy grass was very effective in treating hay fever from other grasses as well (27). The same conclusions were reached in the United States concerning the temperate pasture grasses (subfamily Pooideae), but the uniqueness of bermuda grass was appreciated by the early 1920s *(28,29).* More recently, Leavengood and colleagues reported that allergen immunotherapy with timothy and bermuda alone resulted in significant skin-test diminution not only to those two grasses, but june, smooth brome, quack, johnson, bahia, grama, and salt grasses as well *(30).* Bahia grass differs by skin-test comparisons and challenges from timothy (31).

Several RAST inhibition studies utilizing crude extracts demonstrated that members of the fescue subfamily of grasses strongly cross-react with only minor exceptions *(32–34).* Bernstein and coworkers demonstrated tight inhibition curves between meadow fescue, june, ryegrass, orchard, timothy, and sweet vernal, while common reed appeared to contain different allergens and was a poor inhibitor of the others *(32).* Bermuda was a poor inhibitor but appeared to have distinct allergens. Leiferman and Gleich showed that meadow fescue, june, orchard, and ryegrass were equally effective in inhibition, while timothy was less so, but appeared to have unique allergens *(33).* Bermuda and sweet vernal were similarly less effective and did not appear to contain unique allergens, but the authors commented that these grasses were not endemic in Minnesota, and the patient serum pool may not have recognized unique allergens. Martin and associates investigated western as well as northern and southern grasses *(34).* They found similar results with the temperate pasture grasses, with western wheatgrass and quackgrass also inhibited by timothy and fescue. Quackgrass inhibited western wheatgrass but not vice versa. Bermuda, grama, and salt grass were cross-inhibitory, with bermuda being the most potent. Johnson and bahia grasses were inhibited by the northern grasses, but were poor inhibitors themselves. Muggelberg and colleagues examined by ELISA inhibition several western prairie grasses to include cheat, canary, creeping bent, cultivated rye, redtop, sweet vernal, velvet, and indian grass *(35).* All are members of the Pooideae subfamily, with the exception of indian grass, which is in Panicoideae. All showed strong cross-allergenicity, with canary grass being the most potent inhibitor. Indian grass did not cross-react to any great degree. González and coworkers demonstrated similar results using RAST inhibition, immunoblots, and CRIE *(36).* They showed cross-reactivity between Bermuda and other grasses, but only with minor allergens. Despite extensive reactivity among temperate pasture grasses, it is possible to raise MAbs

Table 3
Purified Grass Pollen Allergens

Allergen	Molecular weight (kD)	% Patient reactivity	Comments
Group 1	27–32	95%	Papain-like cysteine protease (β-expansin)
Group 2/3	19–20	60%	
Group 4	50–60	70%	pI = 9.0–9.3
Group 5 (old Grp 9)	31–35	70–80%	
Group 6	10–15		
Group 7	8–12	10%	Calcium-binding protein
Group 10	30		Cytochrome C
Group 11	16	>65%	Soybean trypsin inhibitor-related
Group 12	14	20%	Profilin
Group 13	55–60		Polygalacturonase

with much narrower specificity. Lovborg and colleagues raised MAbs to *Lolium perenne* that reacted to *Festuca elatior*, but not to *Phalaris arundinacea* (reed canary grass) or other Poeae members *(37).*

Pollens from the cereal grain grasses have also been investigated *(38).* Cereal rye, wheat, and barley showed similar levels of weak inhibition of grass pollen, whereas oat appeared somewhat different, and corn showed little cross-reactivity. Rice pollen showed no cross-reactivity with corn pollen, and weak cross-allergenicity with wheat, orchard, and timothy pollens *(39).*

Early studies with partially purified allergens supported the crude-extract data. Tangen and Nilsson isolated 30 kD allergens from fescue, ryegrass, timothy, and common reed *(40).* A second allergen of 19 kD was found in all but common reed. Several groups of antigens have been characterized based on molecular weight and isoelectric motility. In 1966, Marsh and associates isolated two groups of major allergens from perennial ryegrass, and his group ultimately identifying third and fourth groups of antigens *(41).* While at times there were over 10 groups designated, several of these have been consolidated. For example, groups 2 and 3 are now considered together, and group 9 has been reclassified into group 5 (*see* Table 3). Major grass allergens are groups 1, 2/3, and 5, with minor allergens in other groups, although reactivity to group 4 allergens has been reported in up to 75% of grass-allergic patients *(42).* Group 1 allergens have recently been shown to be β-expansins, papain-related cysteine proteinases, which catalyse long-term extension of plant cell walls *(43).*

Epitope homology of varying degree has been demonstrated not only between same group allergens of different grass species, but also

between group 1 and 2/3 as well *(44–46).* Work by van Ree and colleagues demonstrated cross-reactive human and rabbit antibodies between groups 1 and 2/3 based on homologies within the C-termini of Lol p 1, 2, and 3 *(46).* Esch and Klapper raised a battery of MAbs to purified group 1 antigens, and demonstrated cross-reactive epitopes as well as unique specificities *(47).* Fahlbusch and coworkers could not show with MAbs cross-reactive epitopes shared between groups 4 and 5 *(48).* Although some epitopes appeared to be shared by all grasses examined, others were not. Epitopes found on the timothy allergen Phl p 5 are shared with ryegrass group 1 allergens *(49).* Matthiesen and colleagues demonstrated homology between the N-terminal sequences of Cyn d 1 and Lol p 1 *(14).* However, Smith and associates reported that a MAb directed against the C-terminal of Lol p 1 did not bind the group 1 homolog of Bermuda (or oat, either) (50). They did find group 1 allergens in both the Pooideae and Panicoideae. They did not, however, find group 5 allergens in other than the Pooideae subfamily, and that some epitopes were not shared outside of the tribe Poeae.

Group 7 calcium-binding proteins are minor allergens, causing reactivity in 10% of grass pollen-sensitive patients. Calcium-induced conformational changes in the molecule give rise to different IgE antibodies *(51).* Bermuda grass Cyn d 7 shows sequence similarity with two other calcium-binding allergens, birch Bet v 4, and oilseed rape Bra r 1, with IgE cross-reactivity shown with the latter allergen *(52).* Lol p 11 is a 16 kD glycoprotein with 32% homology with soybean trypsin inhibitor, although without the enzymatically active site *(53).* There is no sequence homology with other described grass allergens, although a MAb against it did bind with *Dactylis glomerata*, *Festuca rubra*, and *Phleum pratense*. Homology was found with proteins from corn and tomato pollens, and 44% homology with the major olive allergen, Ole e 1. Recombinant Bermuda-grass profilin, Cyn d 12, shows equal IgE reactivity with natural Bermuda-grass allergen, and shares B-epitopes with sunflower profilin *(54).* The relative importance of several of these allergens was investigated by Niederberger and associates by comparing crude extracts with preabsorption with recombinant timothy Phl p 1, Phl p 2, Phl p 5, and birch profilin Bet v 2 *(55).* The human sera pool was comprised of European, American, and Asian subjects. IgE to the four recombinant proteins accounted for a mean 59% of grass pollen-specific IgE. Lower inhibition of IgE binding to the crude extracts of Bermuda grass and corn, *Zea mays*, was attributed to the absence of detectable group 2 and 5 in these two species.

Little is known concerning cross-reactivity among nongrass monocots, although a number have been incriminated in pollinosis, such as palms, sedges, and cattails. Kwaasi and colleagues reported marked differences in the antigenic and allergenic potency of 10 cultivars of

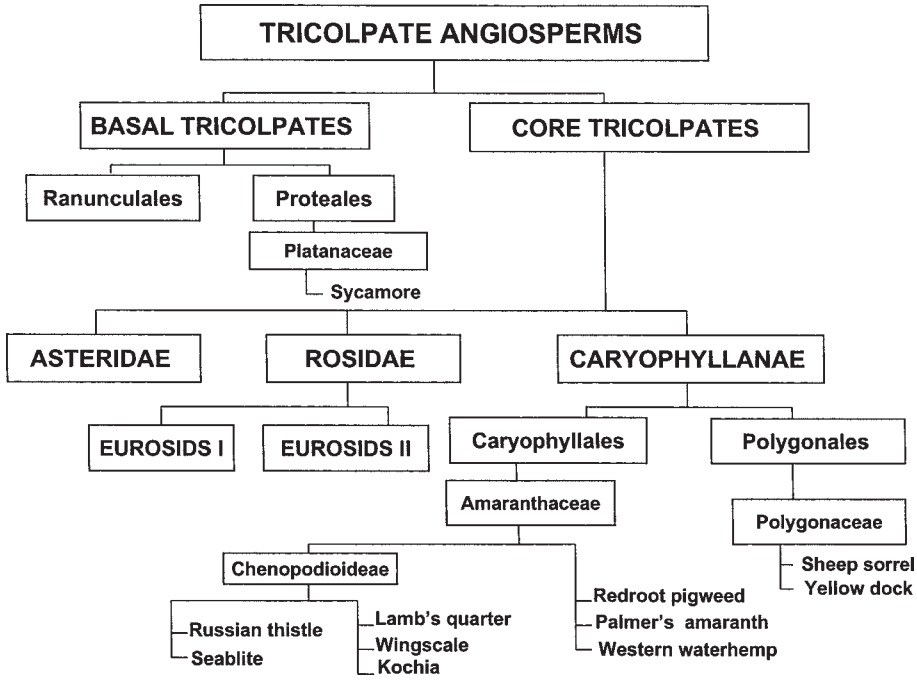

Fig. 5. Taxonomy of the Tricolporate Angiosperm Clade. See text for details.

date palm, *Phoenix dactylifera (56)*. Solomon and coworkers reported preliminary results on reactivity of bulrush, *Scirpus validus*, and two cattails, *Typha latifolia* and *T. angustifolia*, with timothy and perennial ryegrass (57). Fourteen of 22 grass-sensitive patients reacted on skin testing to either or both of the cattails. RAST inhibition showed strong cross-reactivity between the cattails, with little inhibition by timothy. Neither the cattails nor the bulrush could inhibit timothy or perennial ryegrass. Fahlbusch and coworkers found no evidence for group 4 or 5 allergens in sedge (*Carex spp*) *(48)*.

Tricolpate Angiosperms

Three subclades within the tricolpate angiosperm clade (Eudicots) contain the majority of such plants incriminated in pollinosis: subclass Asteridae, subclass Rosidae, and superorder Caryophyllanae (*see* Fig. 5). The latter includes Amaranthaceae and Polygonaceae families (*see* Table 4 for representative members). In older classifications, Chenopod and Amaranth weeds were separate families in a separate order, Chenopodiales. Current evidence suggests inclusion within Caryophyllales, and the Chenopod weeds are now contained within Amaranthaceae, in the Chenopodioideae subfamily, based on chloroplast DNA restriction sites, ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbcL*) sequences, and morphology. The Chenopod-Amaranth weeds

Table 4
Principal Allergenic Plants of the Superorder Caryophyllanae

Order	Family	Genus	Species	Common name
Caryophyllales	Amaran-thaceae	Amaranthus	retroflexus	Redroot pigweed
			palmeri	Palmer's amaranth
			spinosa	Spiny pigweed
			blitoides	Prostrate pigweed
			albus	Tumble pigweed
		Acnida	tamariscina	Western waterhemp
	(Subfamily Chenopo-dioideae)	Cheno-podium	album	Lamb's quarter
			ambrosioides	Mexican tea
			berlandieri	Goosefoot
		Kochia	scoparia	Burning bush
		Bassia	hyssopifolia	Smotherweed
		Sarcobatus	vermiculatus	Greasewood
		Eurotia	lanata	Winterfat
		Allenrolfia	occidentalis	Burroweed
		Salsola	pestifer (kali)	Russian thistle
			soda	Saltwort
		Suaeda	maritima	Seablite
		Dondia	suffrutescens	Alkaliblite
		Atriplex	canescens	Wingscale (shadscale)
			polycarpa	Allscale
			lentiformis	Lenscale
			wrightii	Annual saltbush
			patula	Common orache
			confertifolia	Sheepfat
		Beta	vulgaris	Sugar beet
		Spinacia	oleracea	Spinach
Polygonales	Polygonaceae	Rumex	acetosa	Garden sorrel
			acetosella	Sheep sorrel
			obtusifolius	Broadleaf dock
			orbiculatus	Water dock
			crispus	Yellow dock
		Rheum	officinale	Rhubarb

contain major inducers of pollinosis in the western and Great Plains states, including tumbleweeds, scales (*Atriplex* species), and pigweeds (*Amaranthus* species). The Polygonaceae includes the sorrels, docks, and rhubarb; there is little cross-reactivity data available for this family. A limited P-K study of sheep-sorrel (*Rumex acetosella*) extract showed strong but not complete suppression of the sheep sorrel sites by ragweed, plantain, and timothy, but not the reverse *(58)*. This suggested

that the relevant allergens of sheep sorrel are shared by the other plants as minor allergens, and the presence of a minor unique allergen.

Allergenic similarities among the chenopod-amaranth pollens based on skin testing was reported by Lamson, as well as Grubb and Vaughan in the 1930s *(8,59).* More recently, Weber performed skin-test Spearman rank correlations on 12 members of the family, showing high r values *(10).* Sellers and Adamson utilized P-K extinction to compare four *Amaranthus* species with lamb's quarters (*Chenopodium album*) and Russian thistle (*Salsola pestifer*) *(60).* The amaranth extracts completely inhibited themselves as well as the two chenopods, whereas the latter weeds could not inhibit the amaranths. Russian thistle was more effective in inhibiting lamb's quarter than vice versa. Wodehouse used Ouchterlony plates to examine the cross-antigenicity of rabbit antisera against Russian thistle and Palmer's amaranth against two *Atriplex* weeds, three other chenopods, and four amaranths *(4).* The amaranths showed essentially identical precipitin bands. Similarly, the two *Atriplex* weeds showed identity. Whereas the other chenopods were much more heterogeneous, Russian thistle and burning bush (*Kochia scoparia*) showed a common antigen with the amaranths. Weber and associates examined nine chenopods (including four Atriplexes) and three amaranths by skin-test correlation, passive double immunodiffusion with rabbit antisera, and RAST inhibition *(61,62).* Redroot pigweed and Palmer's amaranth (*A. retroflexus* and *A. palmeri*, respectively) appeared essentially identical by all techniques, with the third amaranth, western waterhemp (*Acnida tamariscina*), demonstrating fewer precipitin bands on Ouchterlony. Allscale, lenscale, wingscale, and annual saltbush (*Atriplex spp*) showed similar RAST inhibition curves and the same antigen precipitin bands, although in varying concentrations. Lamb's quarter contained the greatest number of cross-reacting antigens on immunodiffusion, but was a weak inhibitor on RAST. Burning bush and smotherweed (*Bassia hyssopifolia*) shared an antigen, and had similar patterns on RAST inhibition. Russian thistle was a potent inhibitor on RAST, and appeared to have strong allergens not shared by the other chenopods. Further investigation with CIE, IEF, and SDS-PAGE immunoblots revealed these same chenopod-amaranth weeds to possess between 5 and 12 antigens, ranging from 20–65 kD *(63).* Crosby and coworkers extended these investigations with ELISA inhibition and enzyme-linked immunoblots with both rabbit antisera raised to Russian thistle and patient antisera *(64).* Russian thistle was the most potent inhibitor, with burning bush, redroot pigweed, lamb's quarter, and wingscale demonstrating similar inhibition slopes within a one log range. Rabbit antisera revealed 11 bands with human IgE showing 8. Wurtzen and coworkers examined *A. retroflexus, C. album, K. scoparia*, and *S. pestifer* extracts with CIE, CRIE, and SDS-PAGE immunoblotting

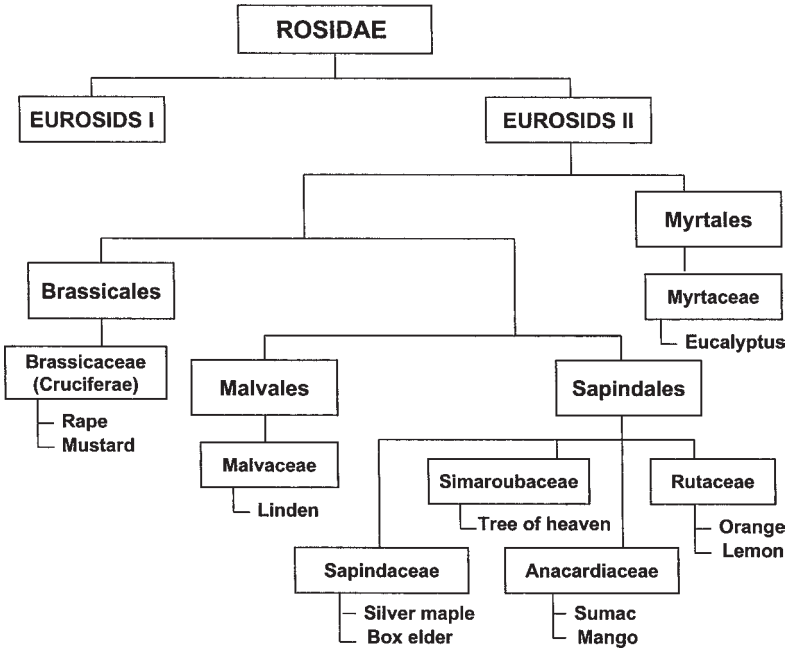

Fig. 6. Taxonomy of the Subclass Rosidae. See text for details.

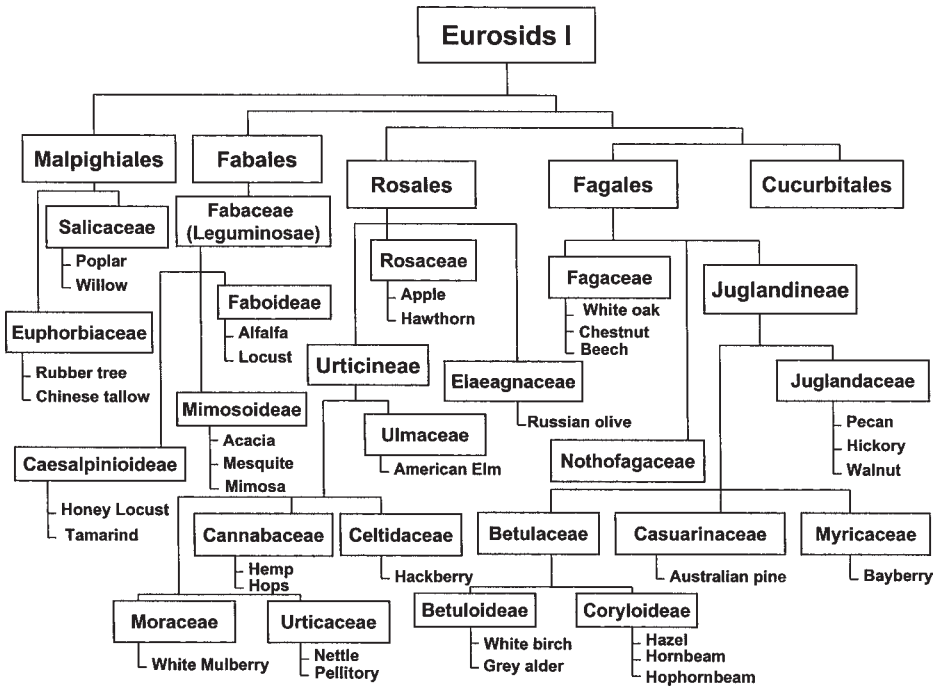

Fig. 7. Taxonomy of the First Rosid Subclade (Eurosids). See text for details.

Table 5
Principal Allergenic Plants of the Subclass Rosidae Group I (Eurosids I)

Order	Family	Genus	Species	Common name
Malpighiales	Salicaceae	Salix	nigra	Black willow
			discolor	Pussy willow
			caprea	Sallow
		Populus	alba	White polar
			deltoides	Common cotton-wood
			nigra italica	Lombardy poplar
			tremuloides	Aspen
			sargentii	Western cotton-wood
			balsamifera	Balsam
	Euphorbiaceae	Hevea	brasiliensis	Rubber tree
		Sapium	sebiferum	Chinese tallow tree
		Mercurialis	annua	Annual mercury
Fabales	Fabaceae (Leguminosae)			
	Subfamily Faboideae	Medicago	sativa	Alfalfa
		Robinia	pseudoacacia	Black locust
	Caesalpin-ioideae	Gleditsia	triacanthos	Honey locust
		Tamarindus	indica	Tamarind
	Mimosoideae	Mimosa	biuncifera	Cat's claw mimosa
		Albizia	julibrissin	Silk tree
		Acacia	dealbata	Mimosa
		Cytisus	scoparius	Scotch broom
		Prosopis	juliflora	Mesquite
Rosales	*See* Table 6			
Fagales	*See* Table 7			

(65). The three techniques gave somewhat disparate results, possibly from denaturation in the SDS medium. Russian thistle appeared to have the greatest number of potent allergens. Two Russian thistle isoallergens of 30 and 42 kD have been isolated *(66).*

The subclass Rosidae splits into two sections, both of which have pollinosis-inducing members. See Figs. 6 and 7, and Tables 5–8 for clarification of taxonomic relationships. The first Rosid subclade (Eurosids I) contains several orders with very important allergenic plants: Rosales, Fagales, Fabales, and Malpighiales. Fabales includes the legume family, Fabaceae (Leguminosae). This family contains a variety of shrubs and trees such as locust (*Robinia spp*), mesquite (*Prosopis juliflora*), and alfalfa (*Medicago sativa*). There is essentially no cross-reactivity data available between family members. Reports of weak

Table 6
Principal Allergenic Plants of the Order Rosales

Order/ suborder	Family	Genus	Species	Common name
Rosales	Rosaceae	Crategus	crus-galli	Cockspur hawthorn
		Malus	pumila	Apple
		Prunus	domestica	Plum
	Elaeagnaceae	Elaeagnus	angustifolia	Russian olive
		Shepherdia	canadensis	Soapberry
Suborder Urticinae	Urticaceae	Urtica	dioica	Stinging nettle
		Laportea	canadensis	Wood nettle
		Boehmeria	cylindrica	Boghemp
		Parietaria	officinalis	Pellitory
		Pilea	pumila	Richweed
	Moraceae	Morus	rubra	Red mulberry
		Broussonetia	papyrifera	Paper mulberry
		Maclura	pomifera	Osage orange
	Cannabaceae	Cannabis	sativa	Hemp
		Humulus	lupulus	Hops
	Celtidaceae	Celtis	occidentalis	Hackberry
			australis	Southern nettletree
	Ulmaceae	Ulmus	americana	American elm
			rubra	Slippery elm
			parvifolia	Chinese elm
			pumila	Siberian elm
			serotina	September elm
			crassifolia	Cedar elm

cross-reactivity between *Acacia* and grasses presumably reflect the presence of strongly conserved proteins, as discussed below.

A major family of Rosales is Rosaceae, containing a large diversity of such fruits as apple, cherry, plum, and pear, as well as almond. These trees are primarily insect-pollinated, but there are numerous reports of pollinosis in nursery or orchard workers. They are of additional interest because of inducing oral allergy syndrome. Hawthorn, again primarily insect-pollinated, is a common sensitizer in some regions. The family Elaeagnaceae contains Russian olive (*Elaeagnus angustifolia*), which has been shown by ELISA inhibition to cross-react with European olive, ash, and privet, members of Oleaceae (subclass Asteridae, order Lamiales)*(67).*

Under older taxonomies, the suborder Urticineae was classified as a separate order, Urticales. It contains elm in Ulmaceae, mulberry in Moraceae, nettle and pellitory in Urticaceae, and hemp and hops in Cannabaceae. Hackberry (*Celtis spp*) used to be included in Ulmaceae,

Table 7
Principal Allergenic Plants of the Order Fagales

Order/ suborder	Family	Genus	Species	Common name
Fagales	Fagaceae	Fagus	grandifolia	American beech
			sylvatica	European beech
		Quercus	alba	White oak
			bicolor	Blue oak
			gambelii	Gambel oak
			macrocarpa	Bur oak
			palustris	Pin oak
			rubra	Red oak
			virginiana	Live oak
		Castanea	sativa	European chestnut
			dentata	American chestnut
Suborder Juglandineae	Juglandaceae	Juglans	regia	English walnut
			nigra	Black walnut
			cinerea	Butternut
		Pterocarya	fraxinifolia	Wingnut
		Carya	illinoensis	Pecan
			ovata	Shagbark hickory
	Myricaceae	Myrica	gale	Sweet gale
			pensylvanica	Bayberry
	Casaurinaceae	Casaurina	equisetifolia	Australian pine
	Betulaceae			
	Sf Betuloideae	Betula	occidentalis	Water birch
			papyrifera	Paper birch
			verrucosa	Silver birch
		Alnus	glutinosa	European alder
			incana	Speckled alder
			viridis	Green alder
	Sf Coryloideae	Corylus	avellana	Filbert
			americana	American hazelnut
		Carpinus	caroliniana	American hornbeam
		Ostrya	carpinifolia	Eur. hophornbeam
			virginiana	Ironwood

Sf, subfamily.

but is now felt to be possibly more closely related to Urticaceae, and has been place in a separate family, Celtidaceae (*see* Fig. 7). Pellitory (*Parietaria spp*) is a major aeroallergenic plant in the Mediterranean basin as well as being found along the California and Gulf coasts. In a European skin test comparison by Serafini, pellitory skin reactivity was quite strong whereas nettle reactivity was very small, suggesting little cross-reactivity between these family members *(68)*. Holgate and col-

Table 8
Principal Allergenic Plants of the Subclass Rosidae Group II (Eurosids II)

Order	Family	Genus	Species	Common name
Myrtales	Myrtaceae	Eucalyptus	globulus	Blue gum
		Melaleuca	quinquenervia	Cajeput
Brassicales	Brassicaceae (Cruciferae)	Brassica	napus	Oilseed rape
			oleracea	Cabbage
			nigra	Black mustard
		Sinapis	alba	White mustard
Malvales	Malvaceae	Tilia	americana	American bass-wood
			cordata	European linden
Sapindales	Rutaceae	Citrus	aurantiifolia	Seville orange
			limon	Lemon
	Simaroubaceae	Ailanthus	altissima	Tree of heaven
	Anacardiaceae	Anacardium	occidentale	Cashew
		Rhus	aromatica	Fragant sumac
		Mangifera	indica	Mango
		Toxicodendron	radicans	Poison ivy
	Sapindaceae	Sapindus	drummondii	Western soapberry
		Acer	rubrum	Red maple
			saccharinum	Silver maple
			platanoides	Norway maple
			macrophyllum	Bigleaf maple
			negundo	Boxelder
		Aesculus	hippocastanum	Horse chestnut
			glabra	Ohio buckeye
		Koelreuteria	paniculata	Golden-rain tree

leagues found similar discordance in British patients *(69)*. Two species of *Parietaria*, *P. officinalis*, and *P. judaica*, showed high degree of identity by CLIE *(70)*.

The order Malpighiales has two families of interest to allergists: Euphorbiaceae and Salicaceae. The former family includes a number of plants with latex-like sap. Annual mercury (*Mercurialis annua*) has been identified as a hay fever-producing plant, especially in the Mediterranean areas of Spain and Italy. The major allergen, Mer a 1, is a profilin and has been cloned. The recombinant protein reacts with sera from patients allergic to annual mercury, olive (*Olea europaea*), and castor bean (*Ricinus communis*) pollens, and shares B-epitopes with sunflower profilin *(71)*. Chinese tallow tree (*Sapium sebiferum*) is a localized inducer of modest pollinosis, but more importantly, *Hevea brasiliensis*, the rubber tree, is the source of natural rubber. The cross-allergenicity of latex proteins with a variety of foods will not be discussed here. How-

ever, latex profilin is highly cross-reactive with ragweed profilin, but does not appear to cross-react with annual mercury profilin *(71,72).* Immunoblot studies by Fuchs and colleagues showed that ragweed, mugwort, and timothy extracts could inhibit IgE binding to latex allergens *(73).* However, Bet v 1 and Bet v 2 (birch profilin) did not appreciably inhibit the latex IgE binding. The role of profilins as panallergens bridging pollen and food sensitivities will be discussed more fully below.

Salicaceae contains cottonwoods, poplars, and aspens (*Populus spp*), and willows (*Salix spp*). Willows are amphiphilous and are perhaps less important in hay-fever production than *Populus* species, which are entirely anemophilous. Appearance of strong cross-reactivity within this family is based on skin test correlations and older work utilizing P-K neutralization *(9,74,75).* Moderate cross-reactivity between Salicaceae and Fagales members is based on P-K neutralization and passive hemagglutination inhibition *(12,76).*

One branch of the order Fagales includes the beech family, Fagaceae, and some smaller families (Fig. 7 and Table 7). Another large branch is the Juglandineae suborder: including most importantly the birch family, Betulaceae; as well as Juglandaceae, Myricaceae, and Casaurinaceae. The latter two families have only minor inducers of pollinosis such as bayberry (*Myrica pensylvanica*) and Australian pine (*Casaurina equisetifolia*). Juglandaceae contains walnut (*Juglans spp*), wingnut (*Pterocarya fraxinifolia*), pecan, and hickory (*Carya spp*). Cross-reactivity data on these family is limited to skin test correlations that show very high r values between shagbark hickory, *Carya ovata*, and pecan, *Carya illinoensis*, and moderate correlations of these former two with black walnut, *Juglans nigra (9).*

Fagaceae members are beech (*Fagus*), oak (*Quercus*), and chestnut (*Castanea*). Betulaceae includes two subfamilies: Betuloideae, with birch (*Betula spp*), and alder, (*Alnus spp*); and Coryloideae with hazel (*Corylus spp*), hornbeam (*Carpinus spp*), and hophornbeam (*Ostrya spp*). Skin test correlations and direct RAST determinations supported the cross-reactivity of the beech and birch families *(9,74,75,77).* P-K neutralization has shown complete cross-desensitization among oak species *(78).* Rackemann and Wagner had shown that birch could completely desensitize oak, maple, and willow sites, but the converse was not true *(12).* Oak was able to desensitize 75% of maple and willow sites, and willow able to desensitize the great majority of maple sites. It appeared that birch was the most potent, containing the relevant allergens of the others with unique allergens as well. There appeared to be varying patterns of shared allergens among the other trees studied. Passive hemagglutination and passive cutaneous anaphylatic (PCA) studies demonstrated modest reactivity between poplar, alder, and oak, with

less between these three and maple and elm, and none with sycamore, olive, or walnut *(76).* RAST inhibition studies by Bernstein and associates revealed cross-inhibition between oaks and other Fagales members, and Zetterström and colleagues showed birch to be a potent inhibitor of other Fagales as well as ash, elm, and willow *(32,79).*

Because birch, alder, and oak are such important aeroallergens in northern Europe, a great deal of work on the purified allergens of the birch and beech families has come from there. N-terminal amino acid sequencing of the major alder allergen, Aln g 1, by Ipsen and Hansen showed partial identity with the corresponding allergens of birch (Bet v 1), hornbeam (Car b 1), and oak (Que a 1) pollens *(80).* Although the major allergen of birch pollen is Bet v 1, an important second major allergen is Bet v 2, a profilin; both of these allergens have been cloned. Recombinant DNA studies showed strong homology between Bet v 1 and these other major allergens *(81).* A recent study reported cross-inhibition of IgE immunoblots by the mix of recombinant Bet v 1 and Bet v 2 *(82).* Homologous inhibition was an average of 92%, whereas inhibition by alder, hornbeam, hazel, and oak were 88, 77, 80, and 72%, respectively. The mix accounted for an average of 82% of the specific IgE, most being directed against rBet v 1. Additionally, cross-reactivity between birch, mugwort, and celery has been demonstrated with Bet v 1, Bet v 2, and higher molecular weight allergens (46–60 kD) *(83).* A 24 kD calcium-binding protein has been identified as Bet v 3 *(84).* A smaller 9 kD calcium-binding protein has been named Bet v 4 *(85).* Bet v 2, Bet v 3, and Bet v 4 are allergenic for 10–20% of birch-sensitive patients. Another allergen, Bet v 5, reactive in about 30% of patients, is an isoflavone reductase-related protein, with cross-reactivity to pear and lychee food allergens *(86).* The group 4 protein from alder, Aln g 4, shares IgE epitopes with similar allergens in tree, grass, and weed pollens *(87).* Recombinant Aln g 2 cross-reacts with an oilseed rape allergen *(88).*

The second Rosid subclade (Eurosids II) accommodates the order Sapindales as well as such diverse families as Myrtaceae, with eucalyptus, Malvaceae with linden, and Brassicaceae (Cruciferae) with oilseed rape and mustard (*see* Table 8). Oilseed rape *(Brassica napus*) is a heavily cultivated turnip-like oilplant. In patients found to be sensitive to rape pollen, concomitant sensitivity is frequently found, especially to grasses. Welch and coworkers recently examined cross-reactivity between grass and oilseed rape in cosensitized individuals using RAST inhibition, and immunoblot inhibition *(89).* They found less than 10% RAST inhibition of solid-phase oilseed rape by the grass extract. Immunoblot studies supported the distinct character of the respective allergens. Two oilseed rape pollen allergens have been identified with weights of 6–8 and 14 kD, and also a cluster of higher molecular aller-

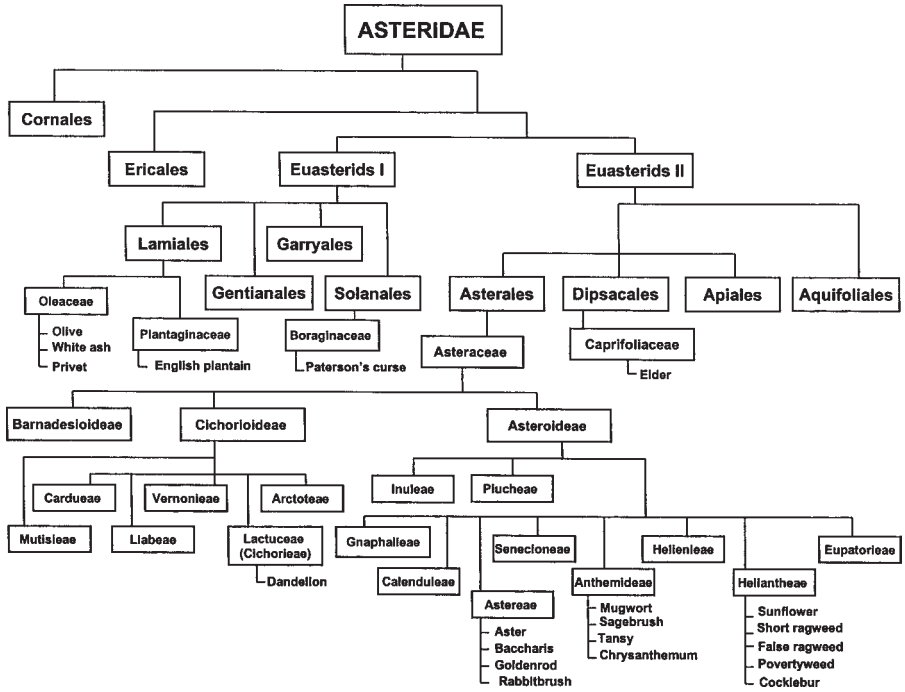

Fig. 8. Taxonomy of the subclass Asteridae, with representative plants of allergenically important families and tribes. See text for details.

gens from 27–69 kD *(88)*. The first two are a calcium-binding protein homologous to Aln g 2 and a profilin, respectively. The larger allergens have IgE-binding carbohydrate moieties, which cross-inhibit timothy group 4 allergens.

Sapindales includes the families Rutaceae with orange and lemon; Simaroubaceae with tree of heaven, Sapindaceae with maple and box elder, and Anacardiaceae with poison ivy, poison sumac, as well as pistachio, cashew, and mango. Maples and boxelder (*Acer spp*) used to be considered members of a separate two-genera family, Aceraceae. Old P-K neutralization data showed cross-inhibition between maple and Fagales members, but RAST inhibition using box elder solid-phase showed poor inhibition with most trees tested, and none with hazel, birch or alder *(12,32)*. This disparity between maple and box elder cross-reactivity may reflect differing techniques, but is also supported by poor skin test correlation between box elder and red maple *(9)*.

The majority of the subclass Asteridae is divided between two groups of "true asters" (*see* Fig. 8 and Table 9). The first group contains the orders Lamiales and Solanales. The latter includes the common Australian weed Paterson's curse, *Echium plantagineum*, in the family Boraginaceae. Baldo and colleagues in several papers have shown skin-

Table 9
Prominent Allergenic Plants of the Subclass Asteridae

Order	Family	Genus	Species	Common name
Euasterids I				
Lamiales	Oleaceae	Olea	europaea	Common olive
		Fraxinus	americana	White ash
			excelsor	European ash
			pennsylvanica	Green ash
		Ligustrum	vulgare	Common privet
	Plantaginaceae	Plantago	lanceolata	English plantain
			majora	Common plantain
Solanales	Boraginaceae	Echium	plantagineum	Paterson's curse
Euasterids II				
Dipsacales	Caprifoliaceae	Sambucus	nigra	Elder
Asterales	Asteraceae	*See* Table 10		

test, RAST, CIE, and CRIE similarities between it and English plantain, *Plantago lanceolata*, in the family Plantaginaceae, order Lamiales *(90,91).* In part, the cross-reactivity is owing to cytochrome c allergens *(92).* In the P-K extinction work by Rackemann and Wagner in 1936, they had shown that both grass and ragweed extracts could neutralize plantain, but the reverse was not true *(12).* This would suggest that the former plants have the relevant allergens of plantain, but that the later does not contain the major allergens of grass or ragweed.

Oleaceae is also in the order Lamiales. It contains both shrubs and trees, including olive (*Olea europaea*), ash (*Fraxinus spp*), privet (*Ligustrum vulgare*), phillyrea (*Phyllyrea angustifolia*), lilac (*Syringa vulgaris*), and forsythia (*Forsythia suspensa*). Olive pollen is an extremely potent aeroallergen in the Mediterranean basin as well as portions of the southwestern states. Ash species have a greater distribution throughout the United States, however. Strong cross-reactivity was demonstrated between family members using RAST inhibition, IEF, and tandem CIE, with olive generally being the strongest inhibitor *(93).* Immunoblotting studies by Baldo and coworkers identified three major allergens of olive at 18–19, 20, and 40 kD, with an additional 70 kD allergen in privet *(94).* RAST inhibition studies show cross-reactivity with these two plants as well as with perennial ryegrass and Bermuda.

At least seven olive allergens have been completely or partially sequenced. Ole e 2 is a profilin, and Ole e 5 is a superoxide dismutase. Obispo and colleagues raised MAbs against the 17–19 kD major allergen of olive, Ole e 1, and showed by CRIE and SDS-PAGE antigenic and allergenic epitope sharing between Ole 1, Fra e 1, Lig v 1, and Syr v 1 *(95).* N-terminal sequencing showed identity of the first 20 amino

Table 10
Principal Allergenic Plants of the Family Asteraceae

Subfamily	Tribe	Genus	Species	Common name
Asteroideae	Astereae	Aster	chinensis	Starwort
		Baccharis	glutinosa	Seep-willow
			halimifolia	Sea-myrtle
		Chryso-thamnus	viscidiflorus	Douglas rabbitbush
		Solidago	gigantea	Goldenrod
	Anthemideae	Anthemis	cotula	Mayweed chamo-mile
		Artemisia	annua	Annual worm-wood
			vulgaris	Common mugwort
			graphlodes	Prairie sage
			frigida	Pasture sage
			tridentata	Giant sagebrush
		Tanacetum	vulgare	Tansy
		Chrysan-themum	leucanthemum	Oxeye daisy
			morifolium	Chrysanthemum
	Heliantheae	Helianthus	annuus	Sunflower
		Parthenium	argentatum	Guayule
		Subtribe		
	Ambrosiinae	Ambrosia	artemisiifolia	Short ragweed
			bidentata	Southern ragweed
			psilostachya	Western ragweed
			trifida	Giant ragweed
			acanthicarpa	False ragweed
			tenuifolia	Slender ragweed
			ambrosioides	Canyon ragweed
			deltoides	Rabbit bush
		Iva	axillaris	Povertyweed
			ciliata	Rough marshelder
			xanthifolia	Burweed marshelder
		Xanthium	communis	Cocklebur
		Dicoria	canescens	Silver ragweed
		Hymenoclea	salsola	Burroweed
Cichorioideae	Lactuceae	Lactuca	serriola	Prickly lettuce
		Taraxacum	officinale	Dandelion

acids between these allergens. Martin-Orozco and associates examined these same four plants as well as forsythia, and found they all had the 18 and 20 Ole e 1 isoallergen homologs with the exception of forsythia, which showed a 50–55 kD cross-reactive protein *(96).* Ash does not share any significant cross-reactivity with the major birch allergen *(97).*

As mentioned earlier, one study has shown strong cross-inhibition between olive, privet, and Russian olive, the latter tree in a different subclass (Rosidae) *(67).*

Ole e 1 has been shown to have a single glycosylation site, which is an IgE-binding epitope *(98).* Antibodies directed against this glycan moiety cross-react with horseradish peroxidase (HRP), bromelain, and ascorbate oxidase. An acidic 9.2 kD calcium-binding protein has been identified as Ole e 3 *(99).* It demonstrates sequence similarities with *Brassica* species pollen allergens, and ELISA inhibition studies show cross-reactivity with a variety of distantly related plants.

Asteraceae (Compositae) is a huge family with 3 subfamilies and 17 tribes (*see* Fig. 8 and Table 10). The majority of members are insect-pollinated and rarely of concern to allergists. The Cichorioideae is paraphyletic, and contains few members that have been incriminated in pollinosis other than dandelion, *Taraxacum officinale*. The subfamily Asteroideae is monophyletic, and contains 10 tribes, of which 3 are of particular interest: the sunflower tribe, Heliantheae; aster tribe, Astereae; and the mayweed tribe, Anthemideae. Ragweeds used to be classified in their own tribe, Ambrosieae, but current data supports incorporating ragweeds and related weeds into a subtribe of Heliantheae, Ambrosiinae. Older taxonomies also divided ragweeds between two genera, *Ambrosia* and *Franseria*. The latter have been reclassified as genus *Ambrosia*. There are numerous species of ragweed. The four major ragweeds are: short, *Ambrosia artemisiifolia*; giant, *A. trifida*; western, *A. psilostachya*; and false *A. acanthicarpa*. Lesser ragweeds are southern, *A. bidentata*, slender, *A. tenuifolia*, and canyon, *A. ambrosioides*. Other members of Ambrosiinae include povertyweed and marshelder, *Iva spp*, and cocklebur, *Xanthium communis* (*see* Table 10). Anthemideae includes mayweed chamomile, *Anthemis cotula*, oxeye daisy, *Chrysanthemum leucanthemum*, and most importantly, mugwort and other sages, *Artemisia spp*. There are numerous native sages in the western states, with introduced mugwort, *Artemisia vulgaris*, found in the eastern states and Pacific Northwest. In addition to mugwort, there are several species found in the European and Asian continents. The aster tribe contains goldenrod, *Solidago spp*, and *Baccharis* species such as sea-myrtle and seep-willow.

The similarity between giant and short ragweed on skin testing was noted as early as 1916 by Cooke and Vander Veer *(100).* Allergenic identity of these two ragweeds was supported by skin test comparisons, P-K neutralization, and extract interchangeability during immunotherapy *(100–104).* Others, however, reported inadequacy of mono-species immunotherapy, skin-test disparities, incomplete P-K neutralization with whole as well as partially purified extracts, and nonidentity of precipitins on Ouchterlony immunodiffusion *(29,105–110).* Cooke and colleagues had felt that some of the differences seen could be explained by

their observation that short ragweed extracts appeared to be 3–10 times more potent than giant ragweed, and that there were not qualitative differences (111).

Bernstein and colleagues compared the four major ragweeds by RAST inhibition, finding then roughly equivalent, with false ragweed being the most effective inhibitor *(32).* Leiferman and associates also studied the major ragweeds by RAST inhibition, in addition to southern and slender ragweeds and other composites *(112).* They found overlapping RAST inhibition curves for short, giant, western, and false ragweeds; slender and southern ragweeds were less potent, and other members of the family showed no inhibition.

Skin-test correlations are moderately strong between Ambrosiinae and Anthemideae members, although not as strong as between the ragweeds themselves *(21,59,111,113).* Prince and Secrest showed by P-K neutralization that marshelder was allergenically distinct from short, giant, and western ragweeds *(114).* Suzuki and coworkers demonstrated that neither ragweed nor goldenrod could desensitize chrysanthemum sites *(113).* Leiferman and associates showed with RAST inhibition that cocklebur (*Xanthium communis*) and annual wormwood (*Artemisia annua*) could weakly inhibit short ragweed, but that other sages (*Artemisia spp*) and burweed marshelder (*Iva xanthifolia*) could not *(112).* Preliminary RAST inhibition data between western ragweed and Santa Maria feverfew, *Parthenium hysterophorus*, suggested unique as well as cross-reactive allergens *(115).* A more recent RAST inhibition study of short and giant ragweed and feverfew using both US and Indian patient sera (to isolate primary sensitization to ragweeds and feverfew respectively) demonstrated marked cross-inhibition *(116).* Examining pollens from several nonragweed Asteraceae members, Løwenstein, using CLIE and CRIE, demonstrated 25–75% shared antigens and 0–2% shared allergens *(117).*

Purification of ragweed allergens has proceeded over the past 30 years *(118–121).*Using CIE and CRIE, Løwenstein and Marsh showed at least 52 antigens in short ragweed, of which 22 bound IgE *(122,123).* At least 7 short ragweed allergens have been characterized or cloned, the most important being Amb a 1 (Antigen E). Most have several isoforms, and there are so many naturally occurring isoelectric forms of Amb a 1, that it should be considered a family of closely related proteins rather than a single molecule *(124,125).*

Differing recombinant forms of Amb a 1 can elicit distinct reactions at both T- and B-cell levels (126). Amb a 1 and Amb a 2 (Antigen K) are 38 kD acidic multichain molecules with several shared IgE binding epitopes as well as unique sites *(127–130).* Almost all clinically sensitive ragweed sufferers will react to Amb a 1 and Amb a 2 (~90–95%); between 10–21% will respond to the lower molecular-weight basic pro-

teins Amb a 3, Amb a 5, and Amb a 6 *(131).* The earlier described 28 kD basic protein Ra4 (BPA-R) has not been cloned *(120).* Certain patients may be able to distinguish between the homologous allergens from short and giant ragweed, Amb a 5 and Amb t 5 (132). T-cell epitope mapping of Amb a 5 and Amb t 5 has demonstrated the importance of reduction of disulfide bridges into free sulfhyfyl groups in order to elicit T-cell hydridoma cross-reactivity between the two related allergens *(133).* The homologous group 5 allergen from western ragweed, Amb p 5, has also been reported *(134).*

Yunginger and Gleich, using double antibody radioimmunoassay, found Amb a 1(AgE) in short, giant, southern, and false ragweed, with minimal amounts in slender ragweed *(135).* No Amb a 1 was found in cocklebur and marshelder (same subtribe), sages and mugwort (different tribe), plantain (different order), or a variety of more distantly related angiosperms. Lee and Dickinson found Amb a 1 in decreasing amounts in pollen extracts from short, western, southern, canyon, slender, giant, and false ragweeds, respectively *(136).* They also found Amb a 1 in cosmos (same tribe Heliantheae) but not in sunflower, narrowleaf marshelder, or spiny cocklebur. Krilis and associates, using MAb-based enzyme immunoassay, found Amb a 1 in a commercial ragweed mix, lesser amounts in an experimental short ragweed extract, but not in a similar false ragweed extract, nor in mugwort, goldenrod, Paterson's curse, timothy, plantain, and perennial ryegrass extracts *(137).*

Using ELISA inhibition and immunoblot inhibition, Katial and associates examined cross-reactivity in nine *Artemisia* species: *A. annua*, *A. biennis*, *A. californica*, *A. filifolia*, *A. frigida*, *A. gnaphlodes*, *A. ludoviciana*, *A. tridentata*, and *A. vulgaris (138).* They found strongly overlapping inhibition curves with *A. tridentata* and *A. biennis* appearing slightly more potent. Inhibition of immunoblots was virtually complete among the nine species. Brandys and colleagues studied giant sagebrush, *A. tridentata*, as well as five European and Asian sages: *A. vulgaris*, *A. annua*, *A. scoparia*, *A. princeps*, and *A. campestris (139).* They also showed strong cross-reactivity, and additionally felt that there was greater similarity between allergens of greater than 25 kD and more heterogeneity among allergens less than 25 kD. Two mugwort allergens have been identified: Art v 1, a 60 kD glycoprotein, and Art v 2, a 35 kD glycoprotein *(140,141).* Hirschwehr and coworkers demonstrated, using mugwort-sensitized patient sera, that the major allergen Art v 1 was shared by short ragweed *(142).* Cross-reactivity with birch profilin Bet v 2 was also found. This does not, however, mean that mugwort has significant Amb a 1. Cross-reactivity of mugwort pollen allergens with food allergens will be discussed below.

Sunflower allergy has been viewed primarily as an occupational hazard, but may be more prevalent than initially suspected. Thirteen

allergens were detected in *Helianthus annuus* pollen extract by Fernandez and associates on immunoblots, with two of 24–25 kD weight reacting with 95–100% of the patient sera *(143).* RAST inhibition and immunoblot inhibition revealed varying degrees of cross-reactivity, with mugwort the greatest, followed by oxeye daisy (marguerite), *Chrysanthemum leucanthemum*, dandelion, *Taraxacum vulgare*, goldenrod, *Solidago vigaurea*, and short ragweed the least. A strongly cross-reactive profilin has been identified as Hel a 2 *(144).*

Pollen and Food Allergen Cross-Reactivity

The association of certain types of hayfever with food allergy has been appreciated for over 50 years, beginning with the link between ragweed sensitivity and melon allergy, and followed with the association of birch pollen and apple, cherry, pear, or peach allergies, grass and potato, and mugwort with celery, carrot, and orange *(145–147).* Food reactions are generally of the oral allergy-syndrome type, with symptoms centered on the oral cavity. Spice allergy has also been linked to pollen sensitivity (148). Hazelnut allergy has likewise been linked to tree- and weed-pollen sensitivity *(149,150).* These patterns have been supported by RAST inhibition studies with crude extracts as well as with purified allergens. Enberg and coworkers used ELISA inhibition and immunoblots to show shared ragweed and watermelon, *Citrullis vulgaris*, allergens *(145).* Immunoblot studies by Vallier and colleagues showed cross-inhibition of 14–16 kD proteins found in celery, mugwort, and birch extracts *(151).* Examining the same three plants, Bauer and associates demonstrated homologs of Bet v 1, Bet v2, and cross-reactivity of higher molecular weight molecules of 40–60 kD *(152).* Immunoblot studies also revealed strong cross-inhibition between melon and plantain, with orchard grass also showing near complete melon inhibition; the converse was not so, melon only partially inhibiting the grass extract (153).

Purified allergen studies have demonstrated the importance of profilins in this syndrome. Profilin is a highly conserved actin-binding protein found in most eukaryotic organisms. Valenta and colleagues had cloned a birch-pollen allergen (Bet v 2) and identified it as profilin *(154).* In addition to other pollens, they also found it in human platelets. Extending these findings, van Ree and coworkers described profilin in perennial ryegrass, and showed anti-profilin IgE in patients with celery and potato food allergy *(147).* The same group also showed that the association between peach and apple sensitivity and grass pollinosis was to profilins and carbohydrate moieties *(155).* However, Pastorello and colleagues have reported that the major allergen of peach, *Prunus persica*, is not birch-related profilin, but rather a 9 kD

lipid-transfer protein *(156).* RAST cross-inhibition between peach and tree pollens from elm, *U. americana*, ash, *F. americana*, and planetree, *Platanus acerifolia*, as been reported *(157).* Apiaceae spices have been shown to contain both profilin (Bet v 2) and Bet v 1, as well as a 60 kD allergen *(148).* Patients with anaphylaxis to chamomile are frequently sensitive to mugwort, and also birch. A study of such patients showed reactivity to Bet v 1 homologs in some, and IgE binding to higher-weight proteins in others; no Bet v 2 sensitivity was detected (158).

Dau c 1, a 154 amino acid peptide of 16 kD with 3 isoforms, has been cloned, showing IgE binding and cross-inhibition with celery Api g 1, and birch Bet v 1 *(159).* High amino acid sequence identities have been shown between Bet v 1, Dau c 1, Api g 1, as well as the major allergens of the fruits apple, Mal d 1, pear Pyr c 1, and cherry Pru a 1. Scheurer and colleagues have investigated the secondary structure of these allergens, pointing out the importance of the "P-loop" region as a common domain of these pollen-related food allergens and pathogenesis related proteins (160). They were able to show the importance of a serine at position 112 for IgE binding and cross-reactivity. Work by this group on the apple allergen, Mal d 1, has shown that strain differences in allergenicity are owing to varying concentrations of the allergen, rather than strain-specific sequence differences *(161).* Fritsch and associates demonstrated cross-reactivity between Mal d 1 and Bet v 1 at the level of T-cell activation, with 15 of 19 Mal d 1 specific clones reacting to Bet v 1, and 8 of 18 Bet v 1 specific clones reacting with Mal d 1 *(162).* A recent report on zucchini-allergic patients revealed cross-reactivity with melon, another member of Cucurbitaceae *(163).* Basis of the cross-reactivity was the presence of zucchini profilin as well as carbohydrate determinants.

Heiss and associates identified the major mugwort allergen, Art v 1, as a 60 kD molecule with cross-reactive analogs in birch, timothy grass, apple, peanuts, and celery *(164).* Small molecular-weight lipid-transfer proteins, identified as major allergens in peach and apple, were found also in mugwort pollen as well as chestnut, *Castanea sativa (165).* Although there was about 50% sequence identity in the N-terminal third of the protein, there was much less IgE binding with the mugwort and chestnut protein than with the fruits, suggesting conformational differences in the allergens.

Becker and colleagues described in 1981 a 26 kD glycoprotein found in tobacco leaves that had allergenic properties as well as activating Hageman factor *(166).* Similar cross-reactive proteins were found in cocoa powder (*Theobroma cacao*), ground coffee (*Coffea arabica*), and short ragweed pollen, albeit in lower concentrations. These latter glycoproteins were also able to activate the Hageman pathway. A major

allergen of Parthenium, Par h 1, is a 31 kD hydroxyproline-rich glycoprotein with strong cross-reactivity to a 50 kD potato tuber lectin *(167).*

Fungal Interrelationships

Cross-reactivity data for molds is rudimentary, owing in large part to a classification system using artificial "form genera." The Class Fungi Imperfecti (Deuteromycetes) is comprised of members with distinct morphology, but many of these represent the asexual life stages of organisms from the other classes of fungi (Ascomycetes, Basidiomycetes, and Zygomycetes). The taxonomy is therefore confusing. Further difficulty is introduced by the propensity of fungal cultures to show antigenic drift. Qualitative and quantitative antigenic differences can arise even under stable growing conditions *(168).*

It is therefore not unexpected that antigenic differences have been demonstrated between Aspergillus species *(169).* Kim and Chaparas utilized fused rocket IE and reciprocal skin testing in sensitized guinea pigs to show marked antigenic similarity between *Aspergillus fumigatus*, *A. fumigatus* var. *ellipticus*, and *A. phialiseptus (170).* The cross-reactivity appeared to reside in glycoprotein and polysaccharide fractions. These authors found less sharing of antigens between *A. fumigatus* and *A. flavus*, and *A. niger*. Arruda and coworkers purified a major allergen of *A. fumigatus*, Asp f 1, and found 95% sequence homology with mitogillin, a 18 kD cytotoxin from *A. restrictus (171).* It was not found in *A. flavus*, *A. niger*, *A. nidulans*, *A. terreus*, *A. glaucus*, *A. clavatus*, or *A. sydowi*. Further work showed mRNA and polymerase chain reaction (PCR) amplification of Asp f 1 specific DNA present only in *A. fumigatus* and *A. restrictus*, suggesting this cytotoxin was a specific virulence factor for *A. fumigatus (172).*

Varying degrees of cross-reactivity have been shown between *Alternaria*, *Stemphylium*, *Curvularia*, and *Stachybotrys (168).* Bonilla-Soto, Rose, and Arbesman in 1961 reported Ouchterlony studies with rabbit antisera against *Alternaria tenuis*, showing shared antigens with *Curvularia*, *Stemphylium*, *Spondylocladium*, and *Helminthosporium*, but not with *Fusarium*, *Hormodendrum* (*Cladosporium*), *Nigrospora*, or *Pullularia* (*Aureobasidium*) *(173).* RAST inhibition and immunoblot studies have shown little to no inhibition between *Aspergillus fumigatus* and *Alternaria tenuis* and *Cladosporium herbarum*, whereas the latter two showed considerable cross-reactivity with similar slopes on the inhibition curves *(174).* The major *Alternaria* antigen, Alt a 1, appears to be as abundant in *Stemphylium* as in *Alternaria (168).*

Alternaria and *Cladosporium* have a number of homologous allergens: Alt a 10 and Cla h 3 are aldehyde dehydrogenase; Alt a 11 and Cla

h 6 are enolase; Alt a 6 and Cla h 4 are acidic ribosomal protein P2 (a highly conserved human SLE antigen); and Alt a 7 and Cla h 5 are *Saccharomyces cerevisiae* protein YCP4 (unknown function) *(175).*

Enolase appears to be a major allergen with about 50% of *Cladosporium* and *Alternaria* sensitive patients reactive to recombinant enolase *(176).* Inhibition studies showed essential identity between the two enolases. *Alternaria* ribosomal P2 protein has been cloned, as has an *Alternaria* IgE-binding fragment homologous to the C-terminal region of *Cladosporium herbarum* heat shock protein 70 *(177).* These are minor allergens.

Nemergut and colleagues used Ouchterlony immunodiffusion in 1977 to examine cross-reactivity among Penicillium species, finding both identity and nonidentity precipitin lines between *P. notatum* and *P. chrysogenum (178).* No cross-reactivity was seen with *P. citrinum*, *P. italicum*, or *P. roqueforti*. Shen and coworkers developed a MAb against the 68 kD *Penicillium notatum* major allergen (Pen n 20), which reacted with four *Aspergillus* species (*A. flavus*, *A. terreus*, *A. fumigatus*, *A. niger*), and two *Penicillium* species (*P. frequentans*, *P. roseopurpureum*) *(179).* The antibody showed no activity against *Alternaria porri*, *Cladosporium cladosporoides*, *Aureobasidium pullulans*, *Fusarium solani*, *Rhizopus arrhizus*, or *Candidia albicans*. MAbs raised against *Penicillium* species and *Aspergillus* species did not show any cross-reactivity with *Alternaria*, *Aureobasidium*, *Cladosporium*, *Curvularia*, *Stachybotrys*, or *Ulocladium (180).* A cross-reactive *Penicillium* 33 kD major allergen was found in *P. citrinum*, *P. notatum*, and *P. brevicompactum (181).* Further work revealed this allergen to be an alkaline serine proteinase also found in *P. oxalicum*, and N-amino acid terminal sequencing revealed homology with the vacuolar serine proteinase from *Aspergillus fumigatus (182).* A major allergen of *Penicillium notatum*, Pen n 13, shares IgE-binding epitopes with the *P. citrinum* allergen, Pen c 1 *(183).* Complementary DNA cloning of Pen c 3 has shown 82.6% amino acid homology with *Aspergillus fumigatus* peroxisomal membrane allergen (Asp f 3) *(184).* Immunoblot cross-inhibition was demonstrated as well.

The allergenic cross-reactivity of yeasts was investigated by Koivikko and coworkers using skin-test correlations as well as rabbit antisera enzyme-immunoassay inhibition *(185).* Although there was marked skin-test concordance between *Candida* species, *Rhodotorula*, *Saccharomyces*, and others, significant cross-reactivity in the immunoassay was only seen between the *Candida* species. Baldo and Baker used immunoblots and RAST inhibition to show the importance of enolase from bakers' yeast, *Saccharomyces cerevisiae*, and its cross-reactivity with *Candida albicans (186).* More recently, cross-reactivity has been demonstrated between *Candida albicans* and *Pityrosporum orbiculare (187).*

Despite apparent identity of mannan antigens of different species of *Candida*, MAbs can be generated with distinct specificity *(188).*

Basidiomycetes include large fungi such as mushrooms and bracket fungi as well as smuts and rusts. They have been implicated in seasonal allergy as well as epidemic asthma. Tarlo and colleagues investigated the allergenicity of the bracket fungi *Ganoderma applanatum* in 1979, and found no cross-reactivity with rabbit antisera to other common molds such as *Alternaria*, *Aspergillus*, *Candida*, *Penicillium*, *Epicoccum*, or *Helminthosporium (188a)*. Lehrer and colleagues have investigated cross-reactivity among Basidiomycetes using rabbit antisera, RAST inhibition, and immunoblot inhibition *(189,190).* Three members of the Basidiomycete order Agaricales (*Coprinus quadrifidus*, *Psilocybe cubensis*, and *Pleurotus ostreatus*) as well as *Calvatia cyathiformis* (order Lycoperdales) show strong cross-reactivity, but with little to no inhibition demonstrated with *Ganoderma meredithae* (Aphyllophorales) or *Pisolithus tinctorius* (Sclerodermatales).

Cross-Reactivity of Animal Allergens

Inhalant allergy to animal products generally divides into sensitivity to arthropod emanations or mammalian danders and urinary products. Arthropod-induced allergy ranges from barely microscopic mites to easily observed insects such as cockroaches. Additionally, reactivity may occur to parenteral exposures such as stings or bites of mosquitoes, flies, ants, bees, or hornets. The animal phylum Arthropoda includes among others the classes Arachnida and Insecta; the former contains spiders and mites, the latter insects.

Mites

The taxonomy of the mite order Acarina has recently been summarized by Arlian, with attention to the Astigmata grouping, containing house-dust, storage, and scabies mites *(191).* The important house-dust mites are *Dermatophagoides pteronyssinus* and *D. farinae*, which are in the family Pyroglyphidae, and superfamily Analgoidea. Another dust mite, *Euroglyphus maynei*, is also in this family. The scabies mite, Sarcoptes scabei, is in the closely related superfamily Sarcoptoidea, family Sarcoptidae. More distantly related storage mites are members of the superfamilies Glycyphagoidea and Acaroidea: *Blomia tropicalis*, family Echimyopididae, and *Lepidoglyphus destructor*, family Glycyphagidae in the former; and *Acarus siro* and *Tyrophagus putresnetiae*, family Acaridae in the latter.

Fourteen groups of house dust mite allergens have been described, and at least five of these have enzymatic activity (*see* Table 11). The

Table 11
Purified Arthropod Inhalant Allergens

Source	Allergen	Mol Wt (kD)	Function
Dust mite	Group 1	24	Cysteine protease
(*Dermatophagoides spp*)	Group 2	14	? Molting protein
	Group 3	28–30	Trypsin
	Der p 4	56	Amylase
	Der p 5	14	
	Der p 6	25	Chymotrypsin
	Group 7	22–28	
	Der p 8	26	Glutathione-S-transferase
	Der p 9	24	Collagenolytic serine protease
	Der p 10	36	Tropomyosin
	Der f 11	98	Paramyosin
	Group 14	190	Apolipophorin-like protein
(*Blomia tropicalis*)	Blo t 13	15–17	Fatty acid binding protein
Cockroach	Group 1	25–37	
(*Blattella germanica*)	Bla g 2	36	Inactive aspartic protease
	Bla g 4	21	Lipocalin
	Bla g 5	23	Glutathione-S-transferase
	Bla g 6	27	Troponin
(*Periplaneta americana*)	Per a 1		(Cr-PII)
	Per a 3	72–78	Arylphorin-like protein
	Per a 7	33	Tropomyosin
Mosquito	Aed a 1	68	Apyrase
(*Aedes aegyptii*)	Aed a 2	37	

major allergens of Group 1 are cysteine proteases, group 3 are serine proteases (trypsin), group 4 amylases, group 6 chymotrypsins, group 8 glutathione-S-transferases, group 9 collagenolytic serine proteases *(192,193).* These are gut-associated, appear to be digestive enzymes, and are found in larger amounts in mite fecal-pellet extracts. Group 1 allergen (Der p 1, Der f 1) has been localized to the fecal-pellet peritropic membrane. Group 2 allergens do not appear to be gut-related, and are not found in increased amount in fecal material.

Antibodies to crude vaccines have shown at least 21 cross-reacting antigens, half of which are allergens for human antisera. Between 80 and 95% of human mite-specific IgE cross-reacts between *D. pteronyssinus* and *D. farinae (192).* Using standardized extracts with CRIE and single radial immunodiffusion, Lind and coworkers compared *D. pteronyssinus*, *D. farinae*, and *D. microceras (194).* They found both shared and unique epitopes, the latter especially with *D. pteronyssinus*. Limited cross-reactivity has been demonstrated in various studies between

Dermatophagoides, *Euroglyphus*, *Blomia* and other storage mites, as well as with the scabies mite *(192,195–197).*

Several epitopes have been described with MAbs for Der p 1 and Der f 1, only one or two of which show cross-reactivity *(198,199).* Similar results were found using recombinant fragments of Der p 1 *(200).* However, a recent study of T-cell activation by purified Der group 1 and Der group 7 allergens showed a large degree of T-cell cross-reactivity between *D. pteronyssinus* and *D. farinae (201). D. siboney* cross-reactivity was examined by Ferrandiz and associates by immunoblot inhibition *(202).* They found strong inhibition between the pyroglyphid mites (*D. farinae*, *D. pteronyssinus*, *D. microceras*, and *D. siboney*), and less with nonpyroglyphid mites (*L. destructor*, *T. putrescentiae*, *A. siro*, and *B. tropicalis*). N-terminal sequence homology of Der s 1, 2, and 3 was higher to Der f and Der m allergens than to Der p allergens. The homology of group 2 allergens was greater than group 1 allergens. These differences between Pyroglyphidae mites and those from other families was mirrored by Garcia-Robaina and coworkers using RAST inhibition, with the single exception that *Chortoglyphus arcuatus* (family Chortoglyphidae, superfamily Glycyphagoidea) was highly cross-reactive *(203).* Park and colleagues investigated *T. putrescentiae* cross-reactivity with ELISA inhibition and immunoblotting *(204).* The *Dermatophagoides* extracts were able to strongly inhibit the *T. putrescentiae* extract, but the converse was not true. The strongest cross-reactivity appeared to be with the group 2 allergen. Chew and coworkers compared *B. tropicalis* to *D. pteronyssinus* by crossed enzyme IE, FAST inhibition, and dot-blot inhibition (205). The maximum inhibition achieved was only 60%. Recombinant Blo t 5 showed low cross-reactivity with its Der p 5 homolog. Another study by Caraballo and associates, however, showed cross-reactivity between rBlo t 5 and rDer p 5, centered on epitopes in the C-terminal region (206).

The predator orchard mite *Hemisarcoptes cooremani*, of the family Hemisarcoptidae and superfamily Hemisarcoptoidea, does not cross-react with house-dust or storage mites based on rabbit antisera immunodiffusion (207). However, cross-reactivity between house dust mites and snails (*Helix spp*) has been described with ELISA and immunoblot inhibition by Guilloux and colleagues *(208).* They suspected tropomyosin and hemocyanin as the likely allergens. Tropomyosin, an actin-binding protein, has been found to be a panallergen from a variety of species of mollusks, crustaceans, mites, and insects *(208–211).* A recently characterized Der f allergen of 98 kD appears to be a major allergen, showing homology to paramyosin, a ubiquitous muscle protein *(212).* Another high molecular-weight allergen (Mag 3) has likewise been described, showing similarity to prawn *Penaeus japonicus* chitinase, with an internal peptide fragment showing 78% homology *(213).*

Cockroaches

There are over 4000 species of cockroaches, but only about 50 are pests. Most of these household pests, such as the American roach, *Periplaneta americana*, and the oriental roach, *Blatta orientalis*, are peridomestic, living in and around homes. Only the German cockroach, *Blatella germanica*, is strictly indoors, not surviving outside. Helm and colleagues compared whole body extracts of Asian cockroach, *Blatella asahinai*, with *B. orientalis*, *B. germanica*, and *P. Americana* using RAST inhibition and radioimmunoblots *(214).* The Asian roach cross-reacted strongly with the closely related German roach, but less so with the other roaches. Harris and coworkers examined five species of cockroaches by ELISA inhibition with both rabbit antisera and IgE, and found both similarities and evidence for unique allergens *(215).*

The major cockroach allergens primarily originate in the gut epithelium, as is true for dust mites; group 1 cockroach allergens, Per a 1 and Bla g 1, are gut-derived serine proteases. At least seven allergens have been characterized, and most have now been cloned (*see* Table 11). Although the great majority of cloned allergens appear to be species-specific, the group 1 allergens have shared epitopes *(216,217).* In addition to sequence homology between Per a 1 and Bla g 1, there was 31% homology with a mosquito digestive protein, ANG12.

Cockroach extract contains tropomyosin, found in mollusks, crustaceans, mites, and some insects. Crespo and associates demonstrated cross-inhibition between German cockroach and boiled Atlantic shrimp, *Pandalus borealis*, using RAST and immunoblot inhibition *(218).* On blot inhibition, the shrimp extract totally ablated the cockroach binding, but the converse was not true. The same group studied the fish nematode, *Anisakis simplex*, with the same techniques, and found partial cross-inhibition with *B. germanica* and red feather mosquito larvae, *Chironomus spp* (used as fish food). Cloned American cockroach tropomyosin, Per a 7, shows 80% homology with other arthropod tropomyosins, but less with vertebrate tropomyosins *(219).*

Biting Insects

IgE-mediated reactions have been reported for a variety of biting insects, to include among others mosquitoes, deer flies, and kissing bugs; however, cross-reactivity data for biting insects is minimal. As mentioned the shrimp food allergen, tropomyosin, has been found in a variety of arthropods, including mites and fruit flies *(210).* Hemoglobin from Chironomid nonbiting midges, which serve as an inhalant aeroallergen source, has been shown to be allergenic *(220).* Additionally, Eriksson and colleagues have shown that larvae of the midges cross-react with shrimp *(221).* Peng and Simons have demonstrated cross-reactivity between mosquito species allergens *(222).*

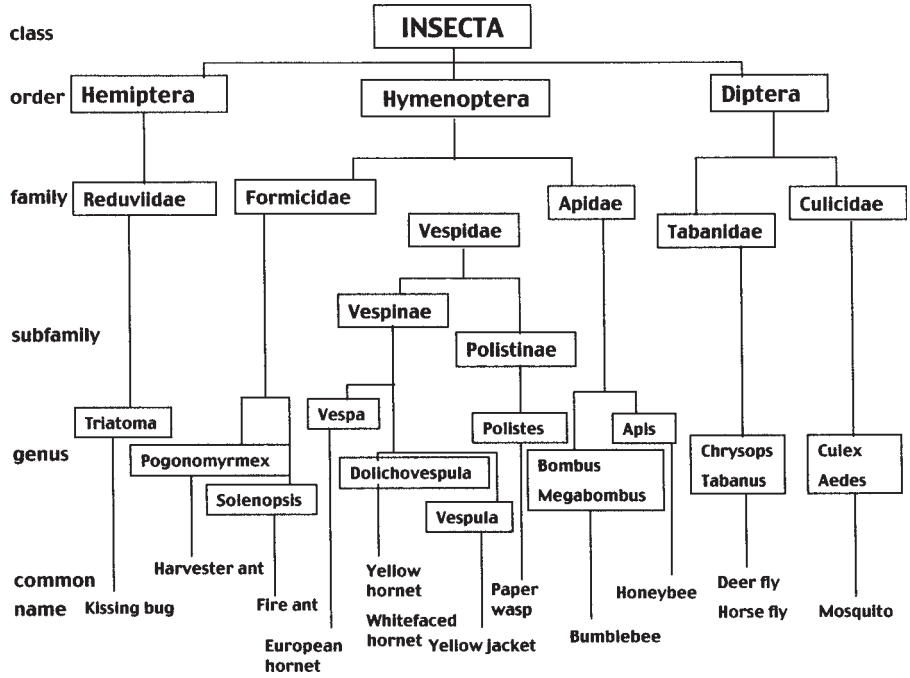

Fig. 9. Taxonomy of stinging and biting insects of the Class Insecta.

Hymenoptera Stinging Insects

Three major families of the order Hymenoptera are the ant family, Formicidae, the bee family, Apidae, and the hornet family, Vespidae (*see* Fig. 9). The latter family has two subfamilies, Vespinae, containing hornets and yellow jackets, and Polistinae, containing wasps. Common European usage calls hornets and yellow jackets wasps, rather than limiting the term to refer to members of Polistinae. Commercially available venoms for skin testing or immunotherapy include: white-faced hornet, *Dolichovespula maculata*; yellow hornet, *Dolichovespula arenaria*; yellow jacket, *Vespula spp*; paper wasp, *Polistes spp*; and honey bee, *Apis mellifera*. Venoms from other Hymenoptera such as bumblebee, *Bombus* and *Megabombus spp*, or fire ant, *Solenopsis spp*, have been studied but are not commercially available at present.

Venoms contain a variety of vasoactive substances, of which phospolipases and hyaluronidases are the major allergens (*see* Table 12). Vespids contain another major allergen, Antigen 5, of uncertain function, although there is sequence homology to tpx, a sperm-coating mouse-testis protein *(223)*. Bee venom does not contain vespid group 5 allergen, but does have a unique protein, melittin, not shared with vespids. It is a small protein with only a single T-cell epitope and a single B-cell epitope, but it has the propensity to form tetramers *(224,225)*.

Table 12
Hymenoptera Venom Allergens

Source	Allergen	Mol Wt (kD)	Function
Honey bee	Api m 1	16	Phospholipase A2
(*Apis mellifera*)	Api m 2	44	Hyaluronidase
	Api m 4	3	Mellitin
	Api m 6	7–8	
Bumble bee	Bom p 1	16	Phospholipase
(*Bombus pennsylvanicus*)	Bom p 4		Protease
Vespids	Group 1	34–35	Phospholipase A1
(*Dolichovespula spp*,	Group 2	44	Hyaluronidase
Vespa spp, *Vespula spp*,	Pol d 4	32–34	Serine protease
Polistes spp)	Group 5	23	Antigen 5
Fire ant	Sol i 2	13	Phospholipase
(*Solenopsis invicta*)	Sol i 3	24	
	Sol i 4	13	
Australian jumper ant	Myr p 1		
(*Myrmecia pilosula*)	Myr p 2		

Hymenoptera show variable cross-reactivity among venoms. Members of the subfamily Vespinae have the strongest cross-reactivity, based on animal antisera studies and RAST inhibition *(226–228).* King and associates found that the cross-reactivity resided primarily in hyaluronidases and antigen 5, with little cross-reactivity seen with the individual phospholipases *(229).* There is also an interrelationship between *Polistes* and Vespinae venoms but not to the same high degree as between yellow jackets and hornets *(230).* Sequence data reinforces this conclusion: hyaluronidase, group 2, is most conserved, followed by group 5, and then group 1; yellow jacket and white-faced hornet are more similar than either is to paper wasp *(231).* A protein similar to antigen 5 has been found in Drosophilia fruit flies *(232).*

Honeybee and vespid cross-reactivity is limited to minor allergens. The assumption that bumblebee venom is similar to honeybee venom is not entirely warranted. A recent study by Stapel and colleagues, utilizing RAST, RAST inhibition, and immunoblotting showed unique allergens not shared by honeybee venom, and also differences between the European bumblebee (*Bombus terrestris*) and American bumblebee (*Megabombus pennsylvanicus*) venoms *(233).*

Venoms from the two imported fire ants, Solenopsis invicta and S. richteri are similar, but S. richteri does not appear to possess group 4 allergen *(234).* Fire ant cross-reactivity with the winged hymenoptera is variable, primarily with yellow jacket *(235).* Sequencing of Sol i 2, 3,

and 4 has been completed by Hoffman *(236).* Sol i 4 and Sol i 2 shared 35% homology; Sol i 3 showed 44–50% homology with vespid group 5 antigens.

Yun and colleagues reported anaphylaxis from stings of Pachycondyla chinensis and Pachycondyla solitaria, ants found in Korea *(237).* RAST inhibition was complete between venom from the two species, with no inhibition by Solenopsis venom. Two small highly basic proteins are major allergens in the venom of the Australian jumper ant, *Myrmecia pilosula*, Myr p 1 and Myr p 2 *(238,239).* The two proteins have an identical C-terminal segment followed by significant differences due to post-translational handling. There are no similarities noted to other protein sequences.

Animal Danders

Aeroallergens from mammalian sources may have several sources: sebaceous and salivary glands, serum, or urine. Ingested allergens will not be discussed here. The major domesticated cat allergen, Fel d 1, is a product of sebaceous and salivary glands and is shared by all cats *(240,241).* Similar cross-reacting proteins have been found in large (jungle) cats as well *(242).* IgE binding was demonstrated to Fel d 1-like substances in large cats, with the exception of caracal; RAST inhibition was strong with lion and tiger, less with snow leopard. Viander and colleagues performed RAST inhibition studies on cat hair and dog dander, saliva, and urine *(243).* They found some cross-reactivity between the dander components, but saliva and urine appeared to be species specific. Although Fel d 1 is not found in other mammals such as dogs, there does appear to be some epitope sharing between major allergens of dog and cat based on immunoblot inhibition (with unique allergens as well)*(244).* The minor allergen, cat albumin, shares cross-reactivity with albumins of other animals, such as dog albumin. Patients with selective cat sensitivity are most likely to be reactive to Fel d 1, whereas a number of patients sensitive to both cats and dogs will be sensitive to albumin *(245).*

Cabanas and associates, using RAST inhibition, pointed out the cross-reactivity between cat, dog, and horse albumin *(246).* However, they found more than half the IgE antibodies were directed against allergens other than albumin. Spitzauer and coworkers cloned dog albumin, which has been found to be extensively IgE cross-reactive with albumins of various animals, to include human albumin *(247).* This is on the basis of highly homologous amino acid sequences ranging between 82.6% to 75.8% in descending order: human, pig, sheep, cow, rat, and mouse. This group, using ELISA inhibition studies, showed recombinant dog albumin extensively cross-reacted with cat and human albumin *(248).*

The major dog allergen Can f 1 is a salivary lipocalin 21 kD ligand-binding protein *(249,250).* The minor allergen Can f 2 is likewise a salivary lipocalin protein, with homology to mouse urinary protein, Mus m 1 *(250).* Rautiainen and associates have shown that the major bovine allergen Bos d 2 is also a lipocalin, produced by the sweat glands, and acting as a pheromone carrier *(251).* They characterized the T-cell reactive sites, which are limited to the conserved sites on the molecule found on other lipocalins allergens as well as human lipocalins *(252).* Using immunoblot inhibition, two deer allergens were found to be cross-reactive with bovine allergens *(253).*

Conclusions

As plant systematics has become more refined, and phylogenetic relationships clarified, it becomes apparent that the basic premises entertained at the onset of this review hold true: cross-reactivity does reflect taxonomy in the very great majority of cases. However, there have been numerous reports of unexpected cross-reactivity between unrelated plants, sometimes remarkably distant ones. These contradictory observations fly in the face of the basic premise mentioned earlier, that taxonomy will dictate cross-reactivity. How is this possible? The great advances in gene sequencing and the cloning of recombinant proteins have led to the understanding that there are many proteins, presumably performing vital functions, which are tightly preserved throughout the evolutionary tree, even from plants to animals. However, if the conserved proteins are identical, they would not be realized as foreign. Perhaps the small differences that exist between these ubiquitous proteins explains why these are frequently minor allergens not reacting in the majority of allergic sera.

Conifers

The members of Cupressaceae are markedly cross-reactive. A single *Juniperus* pollen extract should be suitable for immunotherapy. Members of Pinaceae should be treated separately in the rare instance of clinical importance. There is no significant cross-reactivity between conifers and angiosperms, the minor reactivity is not of clinical relevance.

Grasses and Related Plants

Members of the grass subfamily Pooideae (temperate pasture grasses and cereal grains) are strongly cross-reactive based on marked homology of groups 1, 2/3, 4, and 5, most of which are major allergens. Differences in cross-inhibition probably reflect quantitative differences rather than qualitative ones, although it is possible that timothy and

sweet vernal may contain relevant unique allergens. Testing with and immunotherapy for northern pasture grasses should be adequately covered by one or two members. There is no need for multiple representation. The Panicoideae and Chloridoideae subfamilies show greater diversity. They appear to lack group 2 and group 5 allergens, accounting for their differences with the temperate grasses; members should be tested for and treated with separately. Those members of the subfamily Chloridoideae examined appear to be cross-reactive, with Bermuda grass being very potent, and the appropriate choice to cover other members. The cross-reacting profilins and calcium-binding allergens are minor allergens. Other nongrass families of monocots show reactivity between members, but no appreciable cross-reactivity with grasses.

Tricolpate Angiosperms

There is great diversity among the plants within these subclasses and orders. Lack of cross-reactivity is the rule even sometimes down to the level of tribe. There are exceptions, with significant cross-reactivity across families, commented on later. In Amaranthaceae, allergenic identity is almost complete among the Amaranthus species such as redroot pigweed and Palmer's amaranth, and slightly less so with another member, western waterhemp. In the subfamily Chenopodioideae, there is greater diversity, although there are almost as striking similarities among the *Atriplex* saltbushes and scales. There are varying degrees of cross-inhibition between other chenopods and amaranths, and Russian thistle appears to possess significant unique allergens. *Amaranthus* and *Atriplex* species can be represented by single members, but the other locally relevant members need to be addressed separately.

The order Fagales shows strong cross-allergenicity within the birch family extending across to the beech family as well. Use of the locally prevalent Betulaceae member should cover other famiy members as well. In areas where oaks are more predominant, a *Quercus* would be expected to cover birch as well. Immunotherapy with rBet v 1 and rBet v 2 recombinant allergens could effectively cover Fagales tree-pollen sensitivity. There is scant information concerning other members of the Rosidae subclass. Generally, one member of a family can be expected to be adequate for immunotherapy, although there are exceptions such as nettle and pellitory.

Olive family members are strongly cross-reactive. In areas where European olive is grown, it seems the proper choice to cover. In other areas where ash is prevalent, it should be adequate. Treating with multiple members should not be necessary.

Asteraceae members show variable cross-reactivity. The four major ragweeds (short, giant, western, and false) strongly cross-react, and one or two are quite adequate. In most locales, it would not be

necessary to test or treat with minor ragweeds. However, in areas were other Asteraceae members are common, such as the sages or marshelders, these need to be handled separately. Because *Artemisia* species are so strongly cross-reactive, it is not necessary to skin test or treat with multiple members; a single choice will do. In the Midwest and West common sagebrush (*A. tridentata*) seems reasonable, whereas in the eastern states and Europe, mugwort (*A. vulgaris*) seems most appropriate.

Pollen and Food Allergen Cross-Reactivity

Food-pollen interrelationships are centered on well-conserved proteins that presumably have important functions in plant survival (plant pathogenesis-related proteins or lipid-transfer proteins). Allergens accounting for the strong Fagales cross-reactivity, Bet v 1 and Bet v 2, and their homologs are prominent in the pollen-food association. Art v 1 also appears to play a prominent role.

Fungal Interrelationships

Cross-reactivity between fungal allergens is poorly delineated, although certain patterns exist. There are some associations between Deuteromycetes, with several contradictory reports, especially in the older literature. *Aspergillus* and *Penicillium* species show some cross-reactivity. *Alternaria*, *Stemphyllium*, *Cladosporium*, and *Stachybotrys* have shared allergens. *Candida* shares epitopes with bakers'/brewers' yeast, *Saccharomyces*, and *Pityrosporum*, based on enolases and mannan antigens. Basidiomycetes show variable associations, with the orders Agaricales and Lycoperdales showing strong cross-reactivity.

Arthropods

Pyroglyphid mites (*Dermatophagoides*, *Euroglyphus*) show fairly strong cross-reactivity, with some unique allergens. More distantly related mites show little cross-inhibition. Generally, other mites such as *Blomia* would need to be tested for separately, if extracts were available. The syndrome of dust-mite sensitivity and snail ingestant allergy is explained by the presence of tropomyosin, a fairly broadly dispersed protein, and the major allergen of shrimp. Cockroach allergens, with the exception of Per a 1 and Bla g 1, show sufficient diversity that locally revelant cockroaches should be tested and treated for separately.

Hymenoptera venom allergens show a mixed picture of similarity and differences. True vespids (yellow jacket, yellow hornet, and white-faced hornet) are sufficiently similar that the sensitizing venom would adequately cover positivity noted to the others. It is not necessary to treat for all three if skin tests are positive. Polistes may be sufficiently

different to warrant individual therapy if it was unclear that skin test positivity was owing to cross-reactivity. Honey bee needs to be treated separately, as would bumblebee if venom was available. Fire ant should also be treated individually, despite demonstration of some cross-reactivity with other Hymenoptera.

Animal Danders

Cat dander sensitivity needs to be treated separately. If there is concomitant dog or other mammal sensitivity, an extract containing both Fel d 1 and cat albumin may be preferable. Dog dander likewise needs to be treated separately. Cross-reacting albumin from dog and cat dander may be expected to impact some benefit on other mammal sensitivity.

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